OCT 14 1930

Volume XXX October, 1930 Number 4

SOIL SCIENCE

Editor-in-Chief
JACOB G. LIPMAN

Associate Editor
HERMINIE BROEDEL KITCHEN

Contents

Rate of Loss of Replaceable Potassium by Leaching. O. C. MAGISTAD..... 243

Some Carbon-Nitrogen Relations in Soils. WILBUR R. LEIGHTY AND EDMUND C. SHOREY..... 257

The Indirect Determination of Various Soil Characteristics by the Hydrometer Method. GEORGE JOHN BOUYOUCOS..... 267

Local Variation of Soil Acidity in Relation to Soybean Inoculation. GEORGE Z. DOOLAS..... 273

Seasonal Variation in the Number of Two Species of Rhizobium in Soil. J. K. WILSON..... 289

Interrelation of Nutrients and Soil Reaction on Growth and Inoculation of Alfalfa. M. C. SEWELL AND P. L. GAINEY..... 297

Determination of Carbonates in Soil. C. J. SCHOLLENBERGER..... 307

PUBLISHED MONTHLY
THE WILLIAMS & WILKINS COMPANY
MT. ROYAL AND GUILFORD AVENUES
BALTIMORE, MARYLAND, U. S. A.

Made in United States of America

SOIL SCIENCE

FOUNDED BY
RUTGERS COLLEGE
NEW BRUNSWICK, N. J.

ADVISORY STAFF

Prof. A. W. Blair, Dr. J. S. Joffe, Prof. L. L. Lee, Dr. H. C. McLean, Dr. S. Mattson, Dr. J. W. Shive, Dr. R. L. Starkey and Dr. S. A. Waksman, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

CONSULTING EDITORS

DR. F. J. ALWAY
University of Minnesota, St. Paul, Minn.

PROF. K. ASO
Imperial University, Tokyo, Japan

PROF. C. BARTHEL
Central Agricultural Experiment Station, Experimentalfaltet, Sweden

DR. P. E. BROWN
Iowa State College of Agriculture, Ames, Iowa

PROF. DR. ALBERT DEMOLON
Ministry of Agriculture, Paris, France

DR. H. J. CONN
New York State Experiment Station, Geneva, N. Y.

DR. E. B. FRED
University of Wisconsin, Madison, Wis.

DR. J. E. GREAVES
Utah Agricultural College, Logan, Utah

DIRECTOR ACH. GREGOIRE
Agricultural Experiment Station, Gembloux, Belgium

DR. B. L. HARTWELL
Rhode Island Experiment Station, Kingston, R. I.

DR. D. J. HISSINK
Agricultural Experiment Station, Groningen, Holland

DR. C. B. LIPMAN
University of California, Berkeley, Calif.

DR. BURTON E. LIVINGSTON
Johns Hopkins University, Baltimore, Md.

DR. F. LÖHNIS
University of Leipsig, Germany

DR. T. L. LYON
Cornell University, Ithaca, N. Y.

DR. M. M. MCCOOL
Michigan State College, East Lansing, Mich.

DR. W. H. MACINTIRE
University of Tennessee, Knoxville, Tenn.

DR. E. A. MITSCHERLICH
University of Königsberg, Prussia

PROF. C. A. MOOERS
University of Tennessee, Knoxville, Tenn.

DR. SVEN ODÉN
Central Agricultural Experiment Station, Experimentalfaltet, Sweden

DR. THEO. REMY
Institut für Boden- und Pflanzenbaulehre, Bonn a. Rh.

PROF. G. ROSSI
Royal Agricultural High School in Portici, Naples, Italy

DR. E. J. RUSSELL
Rothamsted Experimental Station, Harpenden, England

DR. O. SCHREINER
U. S. Department of Agriculture, Washington, D. C.

DR. ALEXIUS A. J. DE'SIGMOND
Royal Hungarian Joseph University of Technical Sciences, Budapest, Hungary

DR. J. STOKLASA
Technical High School, Prague, Czechoslovakia

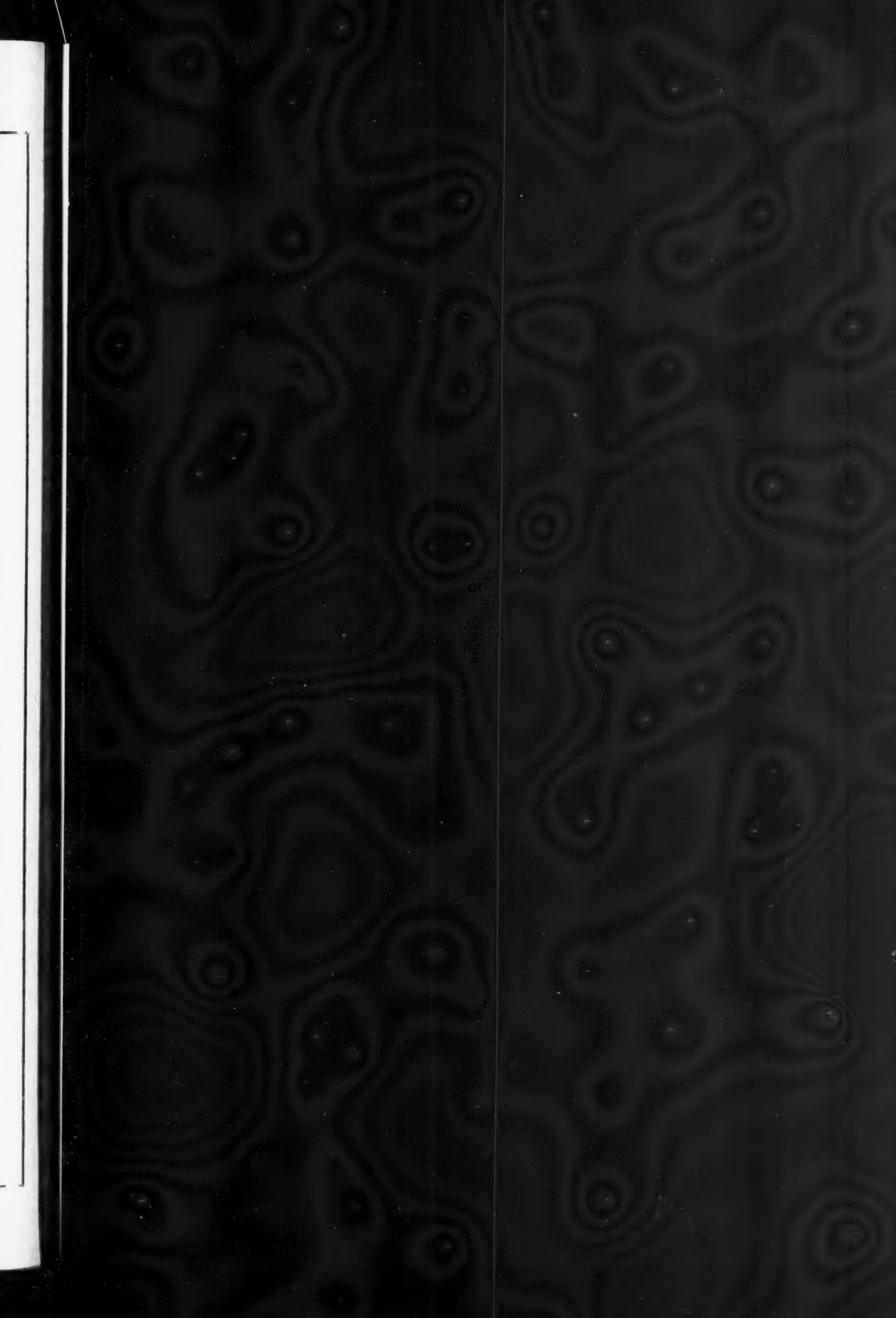
PROF. CHAS. E. THORNE
Ohio Experiment Station, Wooster, Ohio

PROF. N. M. TULAIKOV
Agricultural Experiment Station, Saratov, Russia

DR. F. WEIS
Royal Agricultural and Veterinary College, Copenhagen, Denmark

PROF. S. WINOGRADSKY
Pasteur Institute, Paris, France

Entered as second-class matter May 12, 1919, at the post office at Baltimore, Maryland, under the act of March 3, 1879.
Copyright, 1930, The Williams & Wilkins Company



RATE OF LOSS OF REPLACEABLE POTASSIUM BY LEACHING

O. C. MAGISTAD

Arizona Agricultural Experiment Station

Received for publication January 13, 1930

How rapidly is replaceable potassium lost from a soil by leaching? In the studies of fertilizer practice, of soil deterioration, and of alkali land formation and reclamation it is often desirable to have additional data on this point. Lysimeter experiments have attempted to serve the need, but the results cannot be extrapolated for higher rainfalls nor can they be carried over to other soil types, or even to the same soil when fertilized with potassium salts. This paper records several leaching experiments and shows that for any particular soil the rate of loss can be represented by a mathematical expression.

EXPERIMENTS WITH ARTIFICIALLY PREPARED POTASSIUM ZEOLITE

The plan of the present study was to leach artificial zeolites and soils of known replaceable potassium content with increments of carbon-dioxide-free water. The quantity of potassium removed in each increment was determined and an equation was obtained to fit the experimental data. Because the small amount of work on this subject by earlier authors was expressed by a different mathematical equation than that obtained by the author, all of this material has been reserved for the section "Discussion."

In the first experiments a sample of 1.575 gm. air-dry potassium zeolite was used. This zeolite contained 14.95 per cent K_2O on the air-dry basis and 21.66 per cent on the water-free basis. The sample therefore contained 5 milliequivalents potassium. The zeolite was packed in a small percolation tube between layers of washed and acid-digested asbestos, and leached with distilled, carbon-dioxide-free water at a temperature of about 28°C. Increments of leachate were collected, at first in 25-cc. quantities and later in larger amounts, and titrated for KOH with 0.02 N H_2SO_4, using paranitrophenol as an indicator. Where the increments of leachate were greater than 300 cc. they were concentrated by boiling before titration. The rate of percolation was approximately 50 cc. an hour. The condensed data are given in table 1.

The last three columns of table 1 contain figures used in the mathematical treatment on leaching. This subject will be considered later.

When potassium zeolite is leached the chemical reactions can be represented by the following equations:

$$\text{K zeolite} + \text{HOH} \rightleftarrows \text{H zeolite} + \text{KOH}$$
$$2\text{KOH} + \text{Al}_2\text{O}_3 \rightleftarrows \text{K}_2\text{Al}_2\text{O}_4 + \text{H}_2\text{O}$$

Calculations and data presented in a former paper (11) indicate that more than 99 per cent of the potassium hydroxide arising from hydrolysis of the zeolite is converted into potassium aluminate. A check on the accuracy of the titration can therefore be made by analyzing the solution for aluminum. Such an analysis was made on the combined percolates 21, 22, and 23, and 0.0244 gm. Al_2O_3 was obtained. The titrated value was 24 cc. or 0.48 milli-equivalents. This is equivalent to 0.0245 gm. Al_2O_3. It is thus seen that almost a perfect check was obtained.

TABLE 1

Loss of potassium by leaching from a synthetic zeolite containing 5 milli-equivalents replaceable potassium

NUMBER OF PERCOLATE	CUM. VOLUME	CUM. TITRATION 0.2 N H_2SO_4	y = LOSS K IN MILLI-EQUIVALENTS	$A-y$	$\log(A-y)$	$\log\left(\frac{A}{A-y}\right)$
	cc.	*cc.*				
4	100	8.43	0.168	4.832	0.684	0.015
6	135	10.64	0.213	4.787	0.680	0.019
8	205	14.30	0.286	4.714	0.673	0.026
10	305	19.00	0.380	4.620	0.665	0.034
12	455	24.60	0.492	4.508	0.654	0.045
14	655	30.70	0.614	4.386	0.642	0.057
16	1,000	38.20	0.764	4.236	0.627	0.072
18	1,490	48.60	0.972	4.028	0.605	0.094
20	2,500	68.60	1.372	3.628	0.560	0.139
22	3,500	80.90	1.618	3.382	0.529	0.170
24	4,500	94.60	1.892	3.108	0.492	0.206
26	5,500	105.60	2.112	2.888	0.461	0.238
28	7,500	122.60	2.452	2.548	0.406	0.293
30	9,500	138.00	2.700	2.240	0.350	0.349
32	11,500	151.70	3.034	1.966	0.294	0.405
34	13,500	162.80	3.256	1.844	0.266	0.433
36	15,500	172.60	3.452	1.548	0.190	0.509
38	17,500	180.50	3.610	1.390	0.143	0.556
40	19,500	187.00	3.740	1.260	0.100	0.599
42	21,500	189.40	3.788	1.212	0.083	0.615
44	23,500	192.00	3.840	1.160	0.065	0.635

In the second experiment in which artificial zeolites were used, a quantity equivalent to 10 milli-equivalents replaceable potassium was used. The procedure was similar to that used in the first experiment. A condensed summary of the data obtained is given in table 2.

The data given in table 2 will be treated mathematically under "discussion."

EXPERIMENTS WITH SOILS

In the first experiment with soils a fine sandy loam from the new university farm was used. This soil was first treated with dilute hydrochloric acid to remove all carbonates. It was then leached with a potassium chloride solution

containing a small quantity of potassium hydroxide, followed by potassium chloride alone. The soil was finally washed with water until only a very slight test for chloride in the leachate could be obtained.

The base-exchange capacity of this modified soil was determined and found to be 3.262 milli-equivalents potassium and 0.78 milli-equivalents calcium plus magnesium for each hundred grams.

Five hundred grams of this soil was packed into a small Oldberg percolator and percolated with water using 100- to 150-cc. aliquots. The percolates were analyzed for potassium. The rate of percolation was about 10 cc. an hour

TABLE 2

Loss of potassium by leaching from a synthetic zeolite containing 10 milli-equivalents replaceable potassium

NUMBER OF PERCOLATE	CUM. VOLUME	CUM. TITRATION 0.2 N H_2SO_4	y = LOSS K IN MILLI-EQUIVALENTS	$A-y$	$\log(A-y)$	$\log\left(\frac{A}{A-y}\right)$
	cc.	*cc.*				
3	80	16.20	0.324	9.676	0.9857	0.0143
6	185	23.10	0.462	9.538	0.9795	0.0205
9	337	33.45	0.669	9.331	0.9699	0.0301
12	617	48.95	0.979	9.021	0.9552	0.0448
15	1,267	77.55	1.551	8.449	0.9268	0.0732
18	1,867	98.05	1.961	8.039	0.9052	0.0948
21	2,467	116.65	2.333	7.667	0.8846	0.1254
24	4,000	147.75	2.955	7.045	0.8479	0.1521
27	5,500	177.15	3.543	6.457	0.8100	0.1900
30	7,000	201.35	4.027	5.973	0.7762	0.2238
33	8,500	220.15	4.403	5.597	0.7480	0.2520
36	10,000	236.85	4.737	5.263	0.7212	0.2788
39	12,000	256.15	5.323	4.677	0.6699	0.3301
42	15,000	277.95	5.559	4.441	0.6475	0.3525
45	18,000	302.85	6.057	3.943	0.5958	0.4042
48	21,000	327.55	6.551	3.449	0.5377	0.4623
51	24,000	342.55	6.851	3.149	0.4982	0.5018
54	27,000	360.25	7.205	2.795	0.4464	0.5536
57	30,000	366.45	7.329	2.671	0.4267	0.5733
60	33,000	369.35	7.387	2.613	0.4171	0.5829

until about 800 cc. had passed, when the rate dropped to 30 cc. a day. The data obtained are given in table 3.

In the second experiment a modified Carrington silt loam soil was used. This soil is described as soil 5 in Arizona Agricultural Experiment Station Technical Bulletin 22, and contained 7.13 milli-equivalents replaceable potassium in 100 gm. soil. Five hundred grams of this soil was leached in the same manner as the university farm soil and the leachings were collected and analyzed. The data obtained are given in table 4.

In a third experiment the same modified Carrington silt loam was used as in experiment 2. To it was added precipitated calcium carbonate to equal 2

per cent of its weight. Five hundred grams of this calcareous soil was placed in a percolator and kept in a moistened condition for 2 months before leaching

TABLE 3

Loss of potassium by leaching from a modified soil from the University farm

NUMBER OF PERCOLATE	CUM. VOLUME	y = LOSS K IN MILLI-EQUIVALENTS	A-y	log(A-y)	$\log\left(\frac{A}{A-y}\right)$
	cc.				
1	70	0.77	15.54	1.191	0.021
2	170	1.30	15.01	1.176	0.036
3	278	1.71	14.60	1.164	0.048
4	424	2.02	14.29	1.155	0.057
5	568	2.26	14.05	1.148	0.064
7	859	2.65	13.66	1.135	0.077

TABLE 4

Loss of potassium by leaching from a modified Carrington silt loam

NUMBER OF PERCOLATE	CUM. VOLUME	y = LOSS K IN MILLI-EQUIVALENTS	A-y	log(A-y)	$\log\left(\frac{A}{A-y}\right)$
	cc.				
1	114	1.71	33.94	1.531	0.021
2	210	2.66	32.99	1.518	0.034
3	329	3.34	32.31	1.509	0.043
4	465	3.88	31.77	1.502	0.050
5	597	4.47	31.18	1.494	0.056
6	725	4.89	30.76	1.488	0.064
7	875	5.48	30.17	1.479	0.073

TABLE 5

Loss of potassium by leaching from a calcareous Carrington silt loam

NUMBER OF PERCOLATE	CUM. VOLUME	y = LOSS K IN MILLI-EQUIVALENTS	A-y	log(A-y)	$\log\left(\frac{A}{A-y}\right)$
	cc.				
1	110	0.47	35.18	1.546	0.006
2	220	1.05	34.60	1.539	0.013
3	384	1.61	34.04	1.532	0.020
4	468	2.12	33.53	1.525	0.027
5	589	2.72	32.93	1.518	0.034
6	713	3.27	32.38	1.510	0.042
7	829	3.73	31.92	1.504	0.048
8	959	4.25	31.40	1.497	0.055

was begun. The leachings were collected and analyzed as before. The data so obtained are given in table 5.

The data presented in tables 3, 4, and 5 will be treated mathematically under "Discussion."

DISCUSSION

Schreiner and Failyer in 1906 (17) published a paper describing the absorption of potassium by soils and its subsequent removal by leaching. They apparently imply, but do not seem to state definitely, that absorbed potassium is removed according to the differential equation.

$$\frac{dy}{dv} = K\,(A - y)$$

However, Patten and Waggaman (14), who later carried on similar studies, say, "Thus we have shown that the removal curves for bicarbonate, carbonate, chlorine, potassium, and phosphate leached from soils are very similar in form," and, "The absorption of potassium from carbonate and from chloride solution is likewise described by curves similar in form, and very nearly represented by the formula $\frac{dy}{dv} = K(A-y)$, which also expresses well the rate of absorption and rate of removal of the phosphate radical, PO_4, from soils[1] regardless of what phosphate was used to saturate the soil."

It would thus seem that Schreiner and Failyer's data on removal of potassium by leaching also follow the same differential equation. In this equation, when used to represent leaching data, y is the amount removed by v cc. of water, A is the absorptive capacity and K is a constant. Schreiner and Failyer integrate this differential equation between limits as follows:

$$\int_o^y \frac{dy}{A - y} = K \int_o^v dv \qquad (A)$$

obtaining

$$\log\,(A - y) - \log A = - Kv \qquad (B)$$

They state that since $\log A$ and K are constants to be found from the observations, Briggsian instead of Naperian logarithms may be used. Schreiner and Failyer seem to have obtained the value of A by inspection of the curve obtained by plotting the experimental data.

It was believed that the rate of loss of potassium from artificial zeolites should follow the differential equation (A). Equation (B) indicates that if the values of $\log\left(\frac{A}{A-y}\right)$ given in tables 1 and 2 are plotted as ordinates against the volume in cubic centimeters as abscissas, a straight line whose slope is K should be obtained. Because it is impractical to plot volume as given, log volume was used; equation (B) now becomes

$$\log \log\left(\frac{A}{A - y}\right) = \log K + \log V \qquad (C)$$

[1] (Schreiner and Failyer) Bul. 32, Bur. Soils, U. S. Dept. Agr. p. 36. (Ref. given by Patten and Waggaman.)

To simplify operations log log paper was used and log $\left(\frac{A}{A-y}\right)$ was plotted against V. These data and resulting curves are shown in figure 1. The curve for experiment 1 was a straight line having a slope of 0.695 and for experiment 2, 0.650. The fact that the slope is not 1.00 but a decimal shows that the data do not fit equation (C).

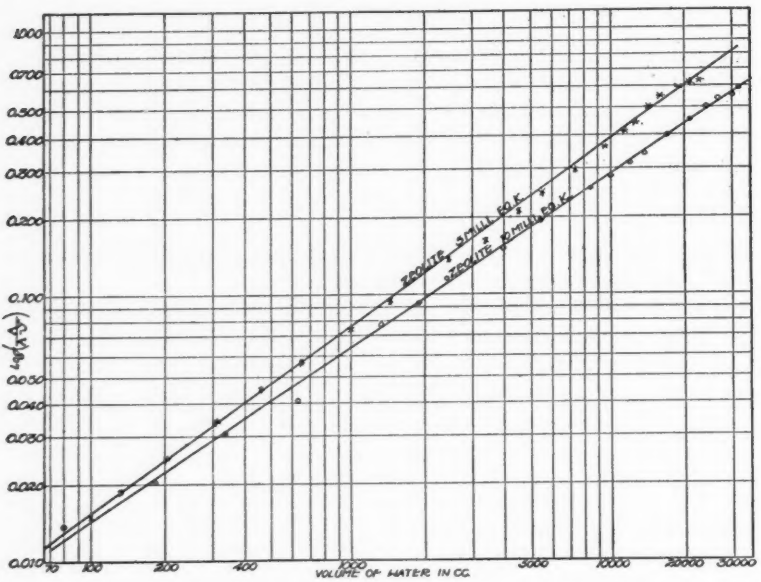


FIG. 1. LOSS OF POTASSIUM BY LEACHING FROM ARTIFICIAL ZEOLITES CONFORMS WITH THE EQUATION

$$\text{Log}\left(\frac{A}{A-y}\right) = KV^{p} \text{ (Value of } p \text{ about 0.65)}$$

Instead, the equations of the curves are

$$\text{Log}\frac{A}{(A-y)} = KV^{0.695} \qquad (D)$$

and

$$\text{Log}\frac{A}{(A-y)} = KV^{0.650} \qquad (E)$$

for experiments 1 and 2 with the artificial zeolites respectively. The values of A and K for equation (D) are 5 and 0.00061 and for equation (E) they are 10 and 0.00071.

In a similar manner the values of log $\left(\frac{A}{A-y}\right)$ from tables 3 and 4 were plotted against the corresponding volumes in cubic centimeters on log log coördinate paper. Both curves were straight lines having slopes of 0.53 and 0.63 respectively (fig. 2). After it is found that the data can be represented by a straight line curve, and the slope of this straight line is known, the values of K

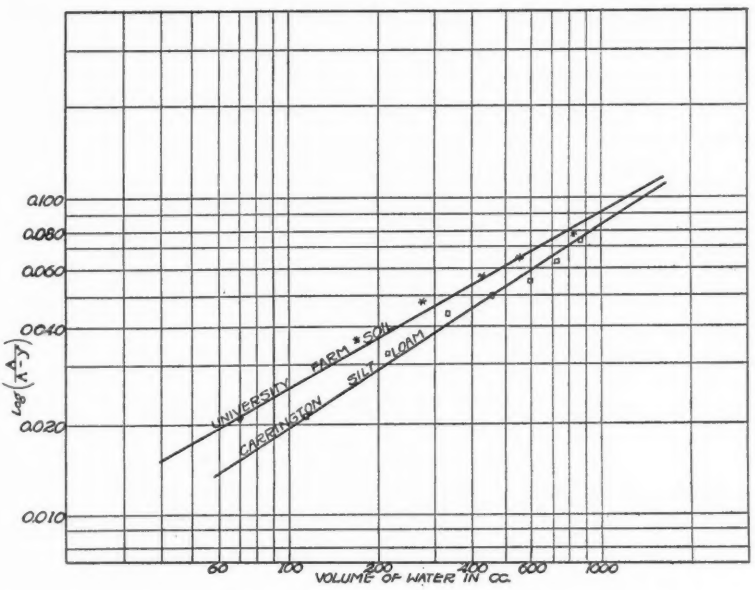


FIG. 2. LOSS OF POTASSIUM BY LEACHING FROM UNIVERSITY FARM SOIL AND CARRINGTON SILT LOAM CONFORMS WITH THE EQUATION

$$\text{Log}\left(\frac{A}{A-y}\right) = KV^{p}$$

can be calculated. It was thus found that the leaching equations for the university farm soil and Carrington silt loam were

$$\text{Log}\left(\frac{A}{A-y}\right) = 0.00219\ V^{0.53}$$

and

$$\text{Log}\left(\frac{A}{A-y}\right) = 0.00100\ V^{0.63}$$

respectively.

It must be remembered that Schreiner and Failyer obtained their value of A from inspection of the absorption curve. The maximum capacity of their soils should have been $A + Y_0$ if we let Y_0 represent the amount of replaceable potassium present before the absorption experiments began. In the author's experiments the maximum exchange capacity of the zeolite in experiment 1 is 7.96 milli-equivalents on the basis of 1 mol K_2O to 1 mol Al_2O_3.

Let us use this value $7.96 = M$[2] where M represents the maximum capacity Let Y_0 equal the loss from this zeolite before the leaching experiment began, or

$$Y_0 = 7.96 - 5.00 = 2.96$$

then $M = A + Y_0$. Integrating the differential equation of Spillman and Lang (18, p. 34)

$$\frac{dY}{dV} = K(M - Y)$$

between the limits as follows:

$$\int_{Y_0}^{Y_1} \frac{dY}{M - Y} = K \int_{V_0}^{V_1} dV$$

we obtain

$$ln\ (M - Y_1) - ln\ (M - Y_0) = - K(V_1 - V_0). \qquad (F)$$

If we let $V = V_1 - V_0$, $Y = Y_1 - Y_0$ and $M = A + Y_0$ and substitute these values in equation (F), we obtain

$$ln\ (A + Y_0 - Y - Y_0) - ln\ (A) = - KV$$

or

$$\ln \left(\frac{A}{A - Y}\right) = KV$$

If we wish to pass to common logarithms the equation becomes

$$\log \left(\frac{A}{A - Y}\right) = K_1 V \qquad (G)$$

where $K_1 = 0.4343K$.

The derivation of equation (G) would indicate that it is perfectly proper to use the quantity of exchangeable potassium at the beginning of leaching as A and to plot the values of $\log \left(\frac{A}{A - y}\right)$ against V, V being the volume of water leached through the sample during the experiment.

[2] The notations used by Spillman and Lang (18), are used as far as possible.

It is possible that the values of A used by Schreiner and Failyer, and by Patten and Waggaman are too small, and that this reduced value of A is responsible for their data giving a slope of 1.00 when $\log\left(\frac{A}{A-y}\right)$ is plotted against volume?

It must be remembered that the value of A used by the aforementioned authors is equivalent to M of equation (F). The fact that the authors did not consider the soil to contain any previously absorbed potassium, and that for the purpose of calculation the value of $A - Y_0$ is less than the amount present at the beginning of leaching would all indicate that their values of A are too small.

WORK OF EARLIER AUTHORS RECALCULATED

Let us recalculate the results of Schreiner and Failyer and of Patten and Waggaman in the light of present knowledge of base exchange. Two assumptions are made, first, that all the potassium present in the soil at the beginning and subsequently removed by leaching was replaceable, and second, that the soil contained normal amounts of replaceable potassium prior to the absorption experiments. The results for the non-calcareous soils are shown in figure 3; the points for any particular soil fall on a straight line curve whose equation is similar to that for preceding soils and zeolites.

The values of A, assumed quantities of replaceable potassium originally present, and of constants in the leaching equations are all given in table 6, which is a recapitulation of all leaching data in this paper.

If we exclude the calcareous soils it will be seen that the leaching equation of the remaining soils has constants of the same order. The leaching equation for zeolites is of the same type but the value of K is somewhat smaller.

Greaves, Hirst, and Lund (5, table 13) present considerable data on the rate of loss of potassium by leaching from synthetic and natural alkali soils. An inspection of their table shows that the potassium is removed in decreasing successive increments and it would appear that the data would fit some sort of logarithmic curve. Because the original amount of exchangeable potassium is not known the data cannot be tested for their conformity with leaching equations as given in this paper.

It should be pointed out that all the leaching experiments for which we have data were short time, excessive leaching, experiments. Under field conditions with more time at our disposal, it is probable that a small quantity of non-replaceable potassium becomes replaceable in the case of soils low in exchangeable potassium. In soils whose exchangeable potassium content has recently been raised, a gradual conversion of some of the replaceable potassium to non-replaceable form seems to take place.

Page and Williams (12) give excellent data on this point and the work of MacIntire and Sanders (10), Pierre and Morley (15), and Frear and Erb (4) corroborates them.

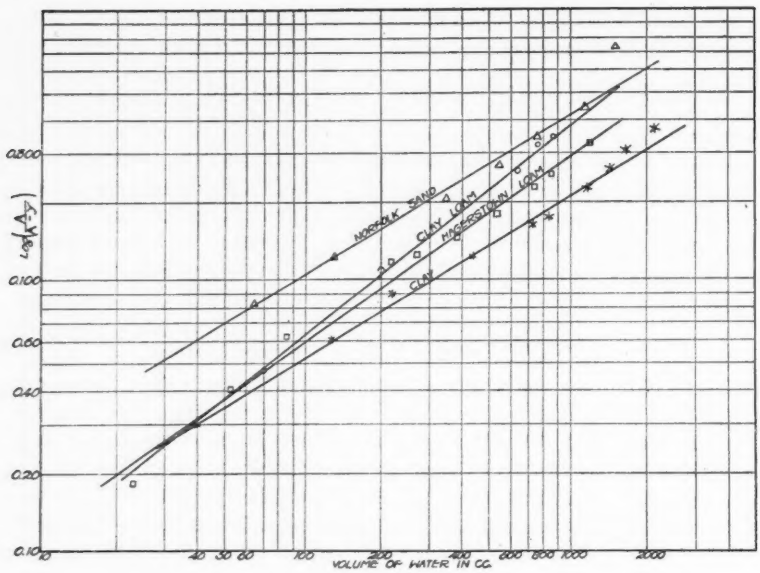


FIG. 3. LOSS OF POTASSIUM BY LEACHING FROM SOILS USED BY SCHREINER AND FAILYER AND BY PATTEN AND WAGGAMAN, AS RECALCULATED BY THE AUTHOR, ALSO CONFORM WITH THE EQUATION

$$\text{Log}\left(\frac{A}{A-y}\right) = KV^p$$

TABLE 6

A comparison of the constants in the potassium leaching equation for soils and artificial zeolites

SOIL	INVESTIGATOR	REPLACEABLE K ASSUMED PRESENT PER 100 GM. SOIL BEFORE ABSORPTION		ASSUMED OR KNOWN VALUE OF A	P EXPONENT OF V	K
		m.eq.	*p.p.m.*			
Artificial zeolite	Magistad	.....	...	5.0 m.e.	0.695	0.00061
Artificial zeolite	Magistad	.....	...	10.0 m.e.	0.650	0.00071
University farm soil	Magistad	.....	...	16.31 m.e.	0.53	0.00219
Carrington silt loam	Magistad	.....	...	35.65 m.e.	0.63	0.00100
Clay	S and F	0.050	20	910.00 p.p.m.	0.60	0.00316
Clay loam	S and F	0.025	10	580.00 p.p.m.	0.69	0.00295
Hagerstown silt loam	P and W	0.100	40	2,974.00 p.p.m.	0.68	0.00263
Hagerstown silt loam	P and W	1.000	400	3,334.00 p.p.m.	0.64	0.00316
Norfolk sand	P and W	0.500	200	1,426.00 p.p.m.	0.58	0.00760
Calcareous soils						
Marshall silt loam	P and W	1.000	400	2,648.00 p.p.m.	1.00	0.00078
Marshall silt loam	P and W	none	none	2,248.00 p.p.m.	0.99	0.00064
Calcareous Carrington silt loam	Magistad	.....	...	35.65 m.e.	1.02	0.00005

It seems that an equilibrium of some sort exists between the potassium containing soil minerals and the soil solution on one hand, and the soil solution and the exchangeable complex on the other. If the amount of exchangeable potassium decreases to such a point that the concentration of potassium in the soil solution is markedly diminished, potassium will go into solution from the soil minerals and vice versa. Data presented in a former paper (11) show a logarithmic relationship between the amount of potassium in the soil solution and that present in exchangeable form, and later studies (1) showed that finely ground feldspar will maintain, to a greater or lesser degree, a certain concentration of potassium in the soil solution.

As a concrete example, let us consider a soil which contains 0.2 milli-equivalent of exchangeable potassium to each 100 gm. and contains 20 per cent of water. The soil solution will probably contain about 41 p.p.m. of K which is in equilibrium with the other soil minerals. This equilibrium can be represented thus:

$$\begin{matrix}\text{Per 100 gm. soil} \\ \left.\begin{matrix}0.2\text{ m.e. K} \\ 20\text{ cc. } H_2O\end{matrix}\right\} \rightleftarrows \text{soil solution} \rightleftarrows \text{Soil minerals} \\ \begin{matrix}\text{Conc. K in} \\ 41\text{ p.p.m.}\end{matrix}\end{matrix}$$

Let us suppose now, that an amount of soluble potassium salt is applied which will approximately double the quantity of exchangeable potassium in the soil. The first part of the foregoing equilibrium expression will then be.

$$\begin{matrix}\text{Per 100 gm. soil} \\ \left.\begin{matrix}0.4\text{ m.e. K} \\ 20\text{ cc. } H_2O\end{matrix}\right\} \rightleftarrows \text{soil solution} \\ \begin{matrix}\text{Conc. K in} \\ 57\text{ p.p.m.}\end{matrix}\end{matrix}$$

Because the soil solution now contains more K, a new equilibrium between the soil solution and the soil minerals will take place. Final equilibrium of the entire system will probably take place according to the following expression.

$$\begin{matrix}\text{Per 100 gm. soil} \\ \left.\begin{matrix}0.36\text{ m.e. K} \\ 20\text{ cc. } H_2O\end{matrix}\right\} \rightleftarrows \text{soil solution} \rightleftarrows \text{Soil minerals} \\ \begin{matrix}\text{Conc. K in} \\ 53\text{ p.p.m.}\end{matrix}\end{matrix}$$

The net result of the fertilizer addition has been to increase the exchangeable potassium 0.16 milli-equivalents to each 100 gm. and the non-exchangeable potassium 0.04 milli-equivalents to 100 gm. This condition of equilibrium is not attained rapidly; undoubtedly several months are necessary.

EFFECT OF $CaCO_3$ ON POTASH LIBERATION

The leaching data for the calcareous soils, the Marshall silt loam and the limed Carrington silt loam, are shown in figure 4. It will be seen that the slope of these curves is about 1.00, which seems to be distinctive for calcareous

soils. A comparison of the leaching data on the calcareous and non-calcareous Carrington silt loams shows that the presence of calcium carbonate has decreased the rate of loss of potassium by leaching. Earlier experiments by the author (11) indicate that this decrease is caused by a decreased hydrolysis. Calcium carbonate in water and the hydrolysis of potassium zeolite both give rise to hydroxyl ions. The common ion will decrease hydrolysis, which may be the principal factor responsible for decreased potassium availability in

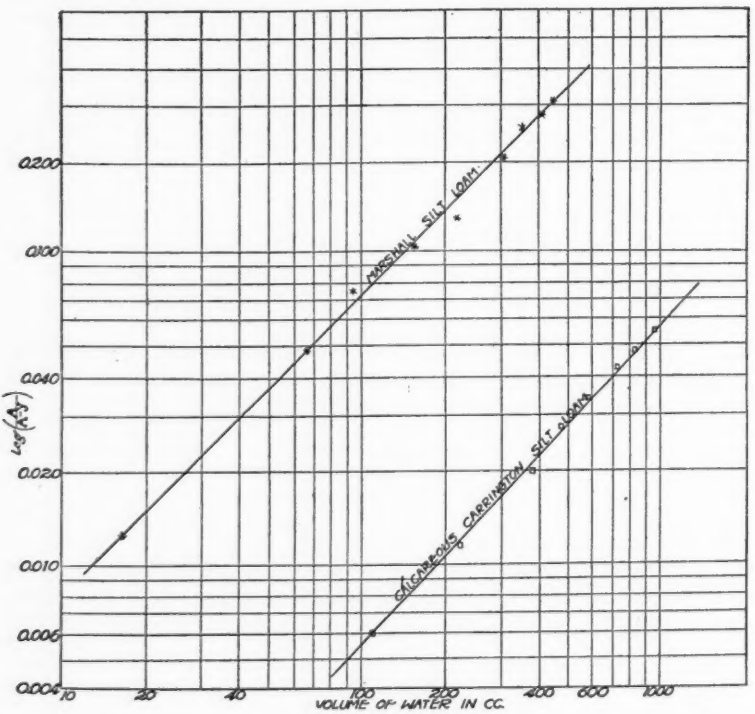


FIG. 4. LOSS OF POTASSIUM BY LEACHING FROM MARSHALL SILT LOAM AS RECALCULATED BY THE AUTHOR, AND CALCAREOUS CARRINGTON SILT LOAM, CONFORM WITH THE USUAL EQUATION BUT WITH A VALUE OF p APPROACHING 1

limed soils. When the solutions were made less alkaline by the introduction of carbon dioxide, the decrease in hydrolysis of potassium zeolite was less marked (11).

A review of the more recent literature indicates that most investigators have found applications of calcium or magnesium carbonates or oxides to decrease the liberation of potash. This is borne out by the work of MacIntire et al. (8, 9), Parker and Tidmore (13), Lyon (6), Plummer (16), Brown and

MacIntire (2), and MacIntire (7). On the other hand, Fraps (3) found that crops of corn, cotton, and sorghum took up slightly more potash from the soil after treatments with calcium carbonate or organic matter than previously.

SUMMARY

Synthetic potassium zeolites were leached with distilled water and the successive portions of leachate analyzed for potassium. The data obtained could be expressed by means of an equation.

Similar studies on a University farm soil and a Carrington silt loam gave rise to a similar equation.

The early work on leaching by Schreiner and Failyer and by Patten and Waggaman has been reviewed. Their data were recalculated in the light of modern base-exchange ideas. It was found that their data did not fit the differential equation

$$\frac{dy}{dv} = K\,(A - y)$$

very well, nor the resulting integral equation

$$\log\,(A - y) - \log A = -Kv$$

The data did seem to agree much better with an equation of the type

$$\log\left(\frac{A}{A - Y}\right) = KV^p$$

where p is a fraction.

The constants in the leaching equation were fairly uniform for the non-calcareous soils. Those for the calcareous soils indicated a much lower rate of loss of potassium, whereas the constants for synthetic zeolites fell between that for calcareous and non-calcareous soils.

Although the constants in the leaching equation for potassium for widely different soils vary considerably, it seems plausible that for a particular series a single set of constants would hold fairly well and that the use of an equation containing these constants would enable one to estimate the rate of loss of potassium over periods of a few years.

REFERENCES

(1) Breazeale, J. F., and Magistad, O. C. 1928 Base exchange in orthoclase. Ariz. Agr. Exp. Sta. Tech. Bul. 24.

(2) Brown, B. E., and MacIntire, W. H. 1911 The relation of certain water-soluble soil constituents in plots 16–24. *Penn. State Col. Ann. Rpt.* 1911: 105.

(3) Fraps, G. S. 1916 The effect of additions on the availability of soil potash, and the preparation of sugar humus. Tex. Agr. Exp. Sta. Bul. 190.

(4) Frear, Wm., and Erb, E. S. 1918 Condition of fertilizer potash residues in Hagerstown silt loam soil. *Jour. Agr. Res.* 15: 59.

(5) GREAVES, J. E., HIRST, C. T., AND LUND, YEPPA 1923 The leaching of alkali soil. *Soil Sci.* 16: 407–426.

(6) LYON, T. L. 1921 The effect of liming on the composition of the drainage water of soils. *Jour. Amer. Soc. Agron.* 13: 124–130.

(7) MACINTIRE, W. H. 1929 The liberation of soil potassium induced by different calcic and magnesic materials, as measured by lysimeter leachings. *Soil Sci.* 27: 337–397.

(8) MACINTIRE, W. H., SHAW, W. M., AND YOUNG, J. B. 1923 A 5-year lysimeter study of the supposed liberations of soil potassium by calcic and magnesic additions. *Soil Sci.* 16: 217–224.

(9) MACINTIRE, W. H., SHAW, W. M., AND SANDERS, K. B. 1927 The influence of liming on the availability of soil potash. *Jour. Amer. Soc. Agron.* 19: 483–505.

(10) MACINTIRE, W. H., AND SANDERS, K. B. 1929 The relation between the absorbed and the exchangeable calcium and magnesium content of a soil four years after additions. *Soil Sci.* 28: 289–304.

(11) MAGISTAD, O. C. 1928 The hydrolysis of sodium and potassium zeolites with particular reference to potassium in soil solutions. Ariz. Agr. Exp. Sta. Tech. Bul. 22.

(12) PAGE, H. J., AND WILLIAMS, W. 1924 Studies on base exchange in Rothamsted soils. *Trans. Faraday Soc.* 20: 573–585.

(13) PARKER, F. W., AND TIDMORE, J. W. 1926 The influence of liming on the phosphate and potash content of the soil solution. *Ala. Agr. Exp. Sta. Ann. Rpt.* 1926: 12.

(14) PATTEN, H. E., AND WAGGAMAN, WM. H. 1908 Absorption by Soils. U. S. Dept. Agr. Bur. Soils, Bul. 52.

(15) PIERRE, W. H., AND MORLEY, S. L. 1928 The buffer method and the determination of exchangeable hydrogen for estimating the amounts of lime required to bring soils to definite pH values. *Soil Sci.* 26: 363–375.

(16) PLUMMER, J. K. 1921 The effects of liming on the availability of soil potassium, phosphorus, and sulphur. *Jour. Amer. Soc. Agron.* 13: 162–171.

(17) SCHREINER, O. AND FAILYER, G. H. 1906 The absorption of phosphates and potassium by soils. U. S. Dept. Agr. Bur. Soils Bul. 32.

(18) SPILLMAN, W., AND LANG, E. 1924 The Law of Diminishing Returns. World Book Co.

SOME CARBON-NITROGEN RELATIONS IN SOILS

WILBUR R. LEIGHTY AND EDMUND C. SHOREY[1]

Bureau of Chemistry and Soils, U. S. Department of Agriculture

Received for publication January 23, 1930

All soils contain organic matter, which in turn contains carbon as an element essential to its organic character, and some of which contains nitrogen in organic combination.

This organic content results from the addition to the soil of the remains of plants, animals, or microörganisms living on or in it. In the changes that take place in the decay of such remains the proportion of carbon to nitrogen becomes less, the carbon disappearing for the most part in the form of carbon dioxide. In the original vegetable or animal remains the proportion of carbon to nitrogen is about 40 to 1, except in the case of leguminous plants with higher nitrogen content, when it may be as low as 25 to 1. In soils, however, it has been commonly held that it is about 10 to 1 and does not vary much; but it has not been apparent just to what extent this assumption of a uniform ratio of 10 to 1 is based on analytical data. Our knowledge of definite nitrogenous organic compounds in soils is not very extensive, but we know that such compounds have a low C:N ratio; for instance, arginine 5.5:1, histidine 2.6:1, xanthine 1.4:1, lysine 2.6:1, cyanuric acid 0.53:1.

In the ordinary fractionation of soil organic matter, whether by acids, alkalis, or organic solvents, it is difficult to obtain fractions that are free from nitrogen. However, on purification by precipitation or repeated solution, a considerable quantity can be obtained nitrogen free; and it is quite apparent that the organic matter of soils is made up of a mixture of compounds—some containing nitrogen and others none; and it might very well be assumed that the proportions of these two classes of compounds would vary in different soils, with a corresponding variation in the C:N ratio.

With a view to determining how far analytical data support the contention, or assumption, that this ratio of C to N in soils is fairly constant, analyses made in these laboratories for other purposes have been reviewed.

The soil samples represent 63 locations in 12 states. In most instances samples at three or more levels were taken, making 172 samples in all.

The samples were taken at points where it was proposed to carry on fertilizer experiments, and the soils were in cultivation at the time the samples were taken. The upper profile of such soils had, of course, been disturbed, but the

[1] Assistant and senior biochemists, respectively, soil fertility investigations.

division between samples was made at depths where color and texture indicated a change from one horizon to another. No horizon designations have been used; however, the depths to which the samples were taken are stated.

The total carbon was determined by combustion in oxygen, and carbon dioxide from carbonates, if present, was deducted from that obtained in combustion, the difference being stated as organic carbon.

The nitrogen was determined by the Kjeldahl method not modified to include nitrates, but should some nitrate appear in the total by the method used, the possible increase would not be significant. A nitrate (NO_3) content of 100 p.p.m. which might be considered excessive, would mean, if it were all recovered, an increase in the total N content of only 0.002 per cent.

The analytical data and calculated C:N ratios are given in table 1. The average C:N ratio given for each location is the average without weighting for variation in the depth to which samples were taken.

The first point apparent on a review of the figures in table 1 is the quite wide variation from the commonly accepted ratio of 10:1. The highest, 35.2:1 was found at the 18- to 36-inch level in the Iberia silty clay loam at Franklin, Louisiana; and the lowest, 3.5:1 at the 24- to 36-inch level in the Bladen fine sandy loam at Baldwin, Florida, with other ratios pretty well spread between these points.

The second point is that in most cases there is a regular lowering of the ratio from the top to the lowest horizon, this being the case in 42 samples out of 46 where three or more depths are represented.

There are 3 samples where the ratio is highest at the lowest depth, and 6 samples where the ratio at the top is three times the ratio at the lowest level. There are 28 locations out of the 76 where the ratio is less than 10:1; 4 where it is 10+:1; and in the remaining 44, 11:1 or above. The average of all the samples without weighting for variation in depth of samples is 10.5:1, and the average of all surface samples is 12.8:1. The average of all samples at 36 inches is 8.2:1.

Other comparisons may, of course, be made, but the figures speak for themselves in opposition to the contention that the proportion of carbon to nitrogen in average American soils is uniform.

In a series of plats at Tifton, Georgia, devoted to green manuring experiments, the analytical data are of interest in this connection. In the plats which receive green manure treatment, the figures for 1928 show a nitrogen content practically uniform, the variation being from 0.03 to 0.04 per cent. The carbon content, although not so uniform, showed no wide variation, being from 0.71 to 1.03 per cent. The C:N ratio varies from 18:1 to 27:1 with an average of 22.7:1. This offers a good example of the ratio being maintained by annual additions of fresh vegetable matter with a ratio probably in excess of 30 or 40 to 1 at a point twice that accepted as the normal.

A further example of extreme variation in the C:N ratio was found in partial analyses of samples of soil from Fontana, California. In these 6 samples the

TABLE 1

Analytical data and calculated C:N ratios of the soil samples studied

LOCATION AND SOIL TYPE	DEPTH	NITROGEN	ORGANIC CARBON	RATIO C TO N-1	AVERAGE C TO N-1
	inches	*per cent*	*per cent*		
Presque Isle, Me., Caribou loam	0–8	0.227	3.00	13.2	10.3
	8–12	0.141	1.55	10.9	
	12–18	0.077	0.77	10.0	
	18–36	0.071	0.50	7.0	
Presque Isle, Me., Washburn loam	0–8	0.320	4.90	15.3	
Allentown, Pa., Berkshale loam	0–8	0.176	1.81	10.2	7.8
	8–18	0.088	0.81	9.2	
	18–36	0.046	0.19	4.1	
Bridgeton, N. J., Sassafras loam	0–10	0.125	1.20	9.6	7.9
	10–20	0.031	0.23	7.4	
	20–36	0.022	0.15	6.8	
Freehold, N. J., Sassafras loam	0–10	0.143	1.66	11.6	8.0
	10–20	0.038	0.33	8.7	
	20–36	0.023	0.09	3.9	
Holmdel, N. J., Sassafras loam	0–12	0.142	1.59	11.1	9.0
	12–18	0.042	0.43	10.3	
	18–36	0.037	0.21	5.6	
Hightstown, N. J., Sassafras gravelly sandy loam	0–10	0.090	0.92	10.2	8.6
	10–18	0.038	0.32	8.4	
	18–36	0.022	0.16	7.2	
Long Island, N. Y., Sassafras sandy loam	0–10	0.116	1.61	13.8	10.6
	10–18	0.054	0.53	9.8	
	18–36	0.032	0.27	8.4	
Cape Charles, Va., Sassafras sandy loam	0–8	0.069	0.86	12.4	8.5
	8–15	0.040	0.31	7.7	
	15–36	0.039	0.22	5.6	
Cape Charles, Va., Sassafras sandy loam, 1921 field	0–7	0.075	0.91	12.1	11.0
	7–16	0.035	0.38	10.8	
	16–36	0.029	0.30	10.3	
Cape Charles, Va., Sassafras sandy loam, 1923 field	0–8	0.065	0.69	10.6	7.3
	8–18	0.026	0.19	7.3	
	18–36	0.023	0.09	3.9	
Bridgetown, Va., Sassafras sandy loam	0–8	0.052	0.51	9.8	7.2
	8–18	0.030	0.14	4.6	
	18–36	0.030	0.22	7.3	

TABLE 1—*Continued*

LOCATION AND SOIL TYPE	DEPTH	NITROGEN	ORGANIC CARBON	RATIO C TO N-1	AVERAGE C TO N-1
	inches	*per cent*	*per cent*		
West Norfolk, Va., Norfolk fine sandy loam	0–9	0.062	0.77	12.4	11.1
	9–20	0.021	0.16	7.6	
	20–36	0.021	0.28	13.3	
Portsmouth, Va., Norfolk fine sandy loam, 1921 field	0–10	0.091	0.65	7.1	6.3
	10–20	0.021	0.16	7.6	
	20–36	0.023	0.10	4.3	
Portsmouth, Va., Norfolk fine sandy loam, 1922 field	0–10	0.106	1.13	10.6	9.8
	10–18	0.028	0.25	8.9	
	18–36	0.025	0.25	10.0	
Suffolk, Va., Bladen fine sandy loam	0–8	0.075	0.87	11.6	8.2
	8–18	0.019	0.14	7.3	
	18–36	0.019	0.11	5.7	
Darlington, S. C., Norfolk sandy loam	0–8	0.026	0.49	18.1	15.4
	8–30	0.013	0.26	20.0	
	30–36	0.025	0.20	8.0	
Darlington, S. C., Norfolk coarse sandy loam, 1919 field	0–8	0.020	0.53	26.5	15.7
	8–20	0.009	0.11	12.2	
	20–36	0.020	0.17	8.5	
Florence, S. C., Norfolk fine sandy loam	0–8	0.033	0.52	15.7	13.9
	8–18	0.010	0.12	12.0	
	18–36	0.024	0.34	14.2	
Florence, S. C., Norfolk very fine sandy loam	0–8	0.049	1.06	21.6	15.3
	8–18	0.016	0.27	16.8	
	18–36	0.024	0.18	7.5	
Darlington, S. C., Portsmouth sandy loam	0–8	0.055	0.80	14.5	11.7
	8–36	0.027	0.24	8.9	
New Bern, S. C., Portsmouth sandy loam	0–12	0.052	0.96	18.5	21.4
	12–36	0.028	0.68	24.3	
Darlington, S. C., Ruston sandy loam 1920 field	0–8	0.033	0.59	17.8	13.4
	8–20	0.012	0.19	15.8	
	20–36	0.015	0.10	6.6	
Fayetteville, N. C., Ruston sandy loam	0–8	0.034	0.77	22.6	15.3
	8–18	0.009	0.14	15.5	
	18–36	0.033	0.26	7.8	
Bennettsville, S. C., Marlboro sandy loam	0–8	0.042	1.05	25.0	15.2
	8–14	0.013	0.19	14.6	
	14–36	0.031	0.19	6.1	

TABLE 1—*Continued*

LOCATION AND SOIL TYPE	DEPTH	NITROGEN	ORGANIC CARBON	RATIO C TO N-1	AVERAGE C TO N-1
	inches	*per cent*	*per cent*		
Lucama, N. C., Marlboro sandy loam	0–5	0.055	0.99	18.0	13.0
	12–18	0.031	0.25	8.0	
Athens, Ga., Cecil sandy loam	0–8	0.051	0.70	13.7	12.9
	8–36	0.027	0.33	12.2	
Shelby, N. C., Cecil sandy loam	0–8	0.034	0.59	17.3	14.4
	8–15	0.027	0.36	13.3	
	15–36	0.021	0.27	12.7	
Gastonia, N. C., Cecil fine sandy loam	0–10	0.041	0.56	13.6	10.3
	10–18	0.033	0.36	10.9	
	18–36	0.018	0.12	6.6	
Lindwood, N. C., Davidson clay loam	0–7	0.066	1.12	16.9	14.8
	7–12	0.055	0.70	12.7	
New Bern, N. C., Dunbar fine sandy loam	0–8	0.065	0.98	15.0	9.6
	8–18	0.020	0.16	8.0	
	18–36	0.027	0.16	5.9	
Fayetteville, N. C., Wickham fine sandy loam	0–7	0.032	0.34	10.6	9.4
	7–14	0.035	0.29	8.3	
Kings Mt., N. C., Appling sandy loam	0–8	0.044	0.93	21.1	14.7
	8–18	0.027	0.34	12.6	
	18–36	0.018	0.19	10.5	
Cozad, Neb., Hall very fine sandy loam	0–10	0.141	1.66	11.7	9.8
	10–18	0.105	1.13	10.7	
	18–24	0.072	0.66	9.1	
	24–36	0.047	0.37	7.8	
Grand Island, Neb., Cass fine sandy loam	0–10	0.148	1.47	9.9	8.0
	10–18	0.069	0.65	9.4	
	18–24	0.024	0.19	7.8	
	24–36	0.021	0.11	5.2	
Rocky Ford, Colo., Las Animas clay	0–8	0.103	0.85	8.2	7.6
	8–18	0.071	0.58	8.1	
	18–24	0.043	0.31	7.2	
	24–36	0.044	0.32	7.2	
Lamar, Colo., Manville silt loam, Center Farm 1923	0–8	0.109	0.97	8.8	8.8
	8–18	0.096	0.90	9.3	
	18–36	0.037	0.31	8.3	
Lamar, Colo., Manville silt loam, Field No. 2, Center Farm 1924	0–10	0.118	1.06	8.9	

TABLE 1—*Continued*

LOCATION AND SOIL TYPE	DEPTH	NITROGEN	ORGANIC CARBON	RATIO C TO N-1	AVERAGE C TO N-1
	inches	*per cent*	*per cent*		
Rocky Ford, Colo., Rocky Ford Loam, Test plots 1924	0–13	0.095	0.90	9.4	
Rocky Ford, Colo., Rocky Ford fine sandy loam, Research plots 1925	0–8	0.128	1.20	9.3	7.2
	8–15	0.105	0.78	7.4	
	15–36	0.049	0.25	5.1	
Avondale, Colo., Rocky Ford fine sandy loam	0–8	0.107	0.88	8.2	7.3
	8–13	0.078	0.62	7.9	
	13–24	0.048	0.34	7.0	
	24–36	0.033	0.21	6.3	
Lamar, Colo., Otero sandy loam	0–10	0.101	1.00	9.9	
Alamosa, Colo., San Luis sandy loam	0–10	0.074	0.56	7.5	5.6
	10–24	0.052	0.28	5.3	
	24–36	0.033	0.14	4.2	
Las Animas, Colo., Ft. Lyon Clay loam	0–9	0.174	1.63	9.4	
Lamar, Colo., Prowers clay	0–8	0.140	1.28	9.1	7.8
	8–18	0.110	0.87	7.9	
	18–24	0.071	0.51	7.1	
	24–36	0.050	0.36	7.2	
Lamar, Colo., Prowers loam	0–9	0.140	1.31	9.3	
Lamar, Colo., Prowers loam	0–9	0.136	1.16	8.5	7.3
	9–18	0.082	0.62	7.5	
	18–36	0.051	0.31	6.0	
Lamar, Colo., Prowers clay loam	0–9	0.136	1.12	8.2	
McClave, Colo., Prowers clay loam	0–9	0.160	1.28	8.0	7.1
	9–20	0.088	0.66	7.5	
	20–36	0.051	0.32	6.2	
Wiley, Colo., Prowers clay loam, 1922 field	0–8	0.150	1.36	9.0	7.2
	8–18	0.080	0.61	7.6	
	18–24	0.052	0.28	5.3	
	24–36	0.042	0.30	7.1	
Wiley, Colo., Prowers clay loam, 1923 field	0–8	0.210	2.01	9.5	7.2
	8–18	0.091	0.76	8.3	
	18–36	0.082	0.32	3.9	
Wiley, Colo., Prowers clay loam, 1924 field	0–9	0.174	1.63	9.3	

TABLE 1—*Concluded*

LOCATION AND SOIL TYPE	DEPTH	NITROGEN	ORGANIC CARBON	RATIO C TO N-1	AVERAGE C TO N-1
	inches	*per cent*	*per cent*		
Salt Lake City, Utah, Jordan sandy loam	0–10	0.134	1.12	8.3	6.8
	10–22	0.066	0.43	6.5	
	22–36	0.052	0.30	5.7	
Franklin, La., Collins silt loam, 1923 Experiment	0–10	0.154	1.74	11.2	8.1
	10–18	0.071	0.56	7.8	
	18–36	0.051	0.28	5.4	
Franklin, La., Collins silt loam, 1925 Experiment	0–10	0.135	1.26	9.3	6.9
	10–18	0.070	0.43	6.1	
	18–36	0.128	0.71	5.5	
Franklin La., Iberia silty clay loam, 1924 Irrigation Experiment	0–9	0.113	1.56	13.8	10.9
	9–18	0.066	0.65	9.8	
	18–36	0.065	0.61	9.2	
Franklin, La., Iberia silty clay loam, 1924 Experiment	0–10	0.125	1.41	11.2	18.3
	10–18	0.078	0.67	8.5	
	18–36	0.048	1.69	35.2	
Franklin, La., Iberia silty clay loam, 1925 Experiment	0–10	0.154	2.01	13.0	10.5
	10–18	0.125	1.53	12.3	
	18–36	0.073	0.46	6.3	
Monticello, Fla., Norfolk sandy loam	0–8	0.041	1.00	24.3	17.3
	8–18	0.031	0.58	18.7	
	18–36	0.033	0.30	9.1	
Cairo, Ga., Norfolk fine sandy loam	0–10	0.056	0.84	15.0	13.5
	10–18	0.026	0.40	15.3	
	18–36	0.029	0.30	10.3	
Pecan City, Ga., Greenville sandy loam	0–10	0.036	0.55	15.2	11.2
	10–15	0.026	0.31	11.1	
	15–36	0.023	0.17	7.4	
DeWitt, Ga., Greenville fine sandy loam	0–8	0.032	0.59	18.4	13.5
	8–15	0.032	0.44	13.7	
	15–36	0.026	0.22	8.4	
Baldwin, Fla., Bladen fine sandy loam	0–6	0.085	1.00	11.7	7.6
	24–36	0.045	0.16	3.5	

N varies from 0.01 to 0.08 per cent and C from 0.11 to 0.79 per cent. The C:N ratio varied from 11.5:1 to 1.8:1, the latter in a soil at the 36-inch level containing 0.06 per cent N and 0.11 per cent C. The water extract of this soil gave no reaction for ammonia, nitrates, or nitrites.

In considering the organic content of soils in relation to soil fertility, it is important to know, not so much the quantity of organic matter, but how much of it is available for supplying food for microörganisms. Since we know that the ratio of carbon to nitrogen in fresh vegetable material is 25:1 or more, and since such material is a good source of food for the microflora of the soil, it would seem that a C:N ratio approaching 20:1 might be considered as indicative of a fair supply of decomposable organic matter and that where the ratio is 10:1 or less, the organic matter is well advanced to the stage where further decomposition will be slow.

This conclusion, however, must be accepted with some reservations. The analytical figures in table 1 show that occasionally there is a high ratio at a low level (36 inches) where this could not be explained by recent additions of fresh organic material, but rather it would appear that such high carbon ratio might be due to an accumulation of organic compounds, the nature of which we, at present, know practically nothing. An inspection of the data, however, shows that abnormally high C:N ratios at lower levels are of rare occurrence.

In considering the relation of the C:N ratio to soil fertility, the ratio alone does not indicate the quantity of decomposable organic matter, since it is possible to have a C:N ratio much greater than 10:1 in the presence of but very small quantities of organic matter. For instance, in the Wickham fine sandy loam, at Fayetteville, North Carolina, with 0.34 per cent C, and a C:N ratio of 16.2:1, the total organic matter calculated by the Van Bemmelén factor is only 0.5 per cent; whereas the Davidson clay loam, from Linwood, North Carolina, with 1.12 per cent C and a ratio of 16.8:1, contains on the same basis three times as much organic matter, viz. 1.64 per cent.

It is evident then, that although a high C:N ratio in a surface soil may be taken as an indication of the presence of organic matter that can be readily decomposed and thereby furnish food for microörganisms, it is no indication of the quantity present.

The calculation of total organic matter in soils is frequently made by multiplying the quantity of carbon dioxide obtained by combustion by the Van Bemmelén factor 0.471 (or C multiplied by 1.724). In view of the fact that soil organic matter is made up of numerous compounds with different carbon content, such calculation can be considered but an approximation and is so regarded where it is intelligently used. It has been proposed to calculate the organic matter by multiplying the nitrogen content by a factor—20 being proposed. In view of the fact that soils contain organic compounds that are nitrogen free and that the nitrogen in those containing nitrogen is variable, the use of such a factor could again be nothing more than an approximation, with the advantage, if any, of eliminating the carbon determination.

A few calculations made from the data in table 1 disclose the fact that there is only occasionally an agreement in the organic content calculated by these two methods. For example:

Iberia silty clay loam Franklin, La.	0–10	N × 20 = 3.08 organic matter C × 1.724 = 3.45 organic matter
Hall fine sandy loam	0–10	N × 20 = 2.82 organic matter C × 1.724 = 2.86 organic matter
Prowers loam Lamar, Colo.	0–9	N × 20 = 2.80 organic matter C × 1.724 = 2.25 organic matter
Prowers clay loam Wiley, Colo.	0–8	N × 20 = 4.20 organic matter C × 1.724 = 3.45 organic matter
Portsmouth sandy loam New Bern, S. C.	0–12	N × 20 = 1.04 organic matter C × 1.724 = 1.65 organic matter
Davidson clay loam Linwood, N. C.	0–7	N × 20 = 1.32 organic matter C × 1.724 = 1.93 organic matter
Caribou loam Presque Isle, Me.	0–8	N × 20 = 4.54 organic matter C × 1.724 = 5.11 organic matter
Sassafras sandy loam Bridgetown, Va.	0–8	N × 20 = 1.04 organic matter C × 1.724 = 0.87 organic matter

Although the figures for Hall fine sandy loam and Sassafras sandy loam are in close agreement, in the others the differences are from 10 to 50 per cent of the total. Since the Van Bemmelén factor is based on a large volume of analytical work and is almost universally recognized as giving an approximation, the calculation of organic matter by multiplying the N by the factor 20 does not appear to have much to recommend it. This is further emphasized by calculating the organic matter N × 20 and then calculating the percentage of carbon in the organic matter from the total organic carbon in the soil.

Portsmouth sandy loam New Bern, S. C.	0–8	N × 20 = 1.04 organic matter Total C = 0.96 which gives organic matter containing 92 per cent C.
Marlboro sandy loam Lucama, N. C.	0–8	N × 20 = 1.10 organic matter Total C = 0.99 which gives organic matter containing 90 per cent C.
Norfolk fine sandy loam Portsmouth, Va.	20–36	N × 20 = 0.46 organic matter Total C = 0.10 which gives organic matter containing 22 per cent C.
Portsmouth sandy loam New Bern, S. C.	12–36	N × 20 = 0.56 organic matter Total C = 0.68 which gives organic matter containing 121 per cent C.

Except for particles of coal, graphite, or charcoal, it is not at all likely that there are organic compounds in soils containing 90 to 92 per cent carbon, and the occurrence of any containing as little as 24 per cent carbon is also unlikely. The figure 121 per cent Portsmouth sandy loam indicate the absurdity of this calculation.

SUMMARY

Analytical data obtained from 176 samples of soil from 63 locations in 12 states show that the C:N ratio is quite variable; and, with a few exceptions, is highest in surface soil and becomes less at lower levels.

It is suggested that some idea of the availability of the organic matter as food for microörganisms may be indicated by the C:N ratio in surface soils.

The data show the futility of attempting to calculate the organic matter by multiplying the total N by the factor 20, as has been proposed.

THE INDIRECT DETERMINATION OF VARIOUS SOIL CHARACTERISTICS BY THE HYDROMETER METHOD[1]

GEORGE JOHN BOUYOUCOS

Michigan Agricultural Experiment Station

Received for publication February 24, 1930

In former communications the hydrometer method has been suggested as a rapid, simple, and reasonably accurate method for determining the total colloidal material in soils in only 15 minutes (3); for determining the total amount of combined sands, silt, and clay or colloids in only 15 minutes (4); and for making a very detailed mechanical analysis of soils (5).

Work is being continued on this method with the idea of improving it, if possible, and also of trying to check it with other methods.

One of the phases of soil physics which has lately been investigated by the hydrometer method consists of ascertaining the relationship that exists between the soil material which the method determines at the end of 15 minutes and designates as the "total colloidal content of soils," and the physical properties or characteristics of soils, such as moisture equivalent, unfree water, and heat of wetting ratio.

EXPERIMENTAL

The relationship that the colloidal material, as determined by the hydrometer method at the end of 15 minutes, has to physical characteristics of soil, has been investigated thus far on three different soil characteristics; namely, heat of wetting ratio (2), moisture-equivalent, and unfree water. Experimental data have already been presented on the first two (3, 6) as well as on their indirect determination. In the present paper the results of a study on the unfree water, and additional data on the moisture equivalent will be presented.

Methods

The procedure consisted of ascertaining first, by the hydrometer method (3), the total colloidal content of the soils to be used in the investigation. Then their moisture equiualent and unfree water content were determined by the new moisture equivalent (6) and dilatometer methods (1) developed in this laboratory. The dilatometer method was somewhat modified in the present work. It consisted of placing 20 gm. of soil, based on oven-dry basis, into the

[1] Published by permission of the director as Journal Article 31 of the Michigan Agricultural Experiment Station.

special 50-cc. dilatometer, adding 10 cc. of water in the case of sandy soils and 15 cc. in the case of clay soils, and stirring the mixture thoroughly by means of a wire. The dilatometer was then filled half full with ligroin of very high boiling point, connected to a bicycle pump and suctioned carefully to eliminate any air in the soil. The dilatometer was then entirely filled with ligroin; stoppered with a cork stopper, the top of which was smeared with tallow; and then placed into an ice bath having a temperature of 0.5°C. The dilatometer was left in this bath, with frequent stirring, until it had completely attained the temperature of the bath and then the reading of the ligroin column on the dilatometer stem was recorded. The dilatometer was then taken out and placed into another ice bath having a temperature of about −10°C.; the reason for transferring from one temperature bath into another, is that at −0.5°C., it is difficult to start solidification of the soil water and it requires a long time for complete solidification to take place, but around −10°C., solidification is rapid, complete, and certain. After the soil water was completely frozen, the dilatometer was transferred again to the original temperature bath of −0.5°C. and allowed to remain there until it attained this original temperature. The ligroin column was again read on the dilatometer stem and from the difference of the two readings the amount of water that failed to freeze at −0.5°C. was calculated. The calculations were based upon the experimental finding by this dilatometer method that 1 cc. of water at 0°C. expands 0.1 cc. upon freezing.

In order that the dilatometer results of the various soils might be comparable, all the soils were washed several times with distilled water for the purpose of eliminating their soluble salts.

Data

In table 1 are shown the amounts of water that failed to freeze at −0.5°C. for a group of representative soils, also their colloidal content and moisture equivalent, and the relationship or ratio that exists between any two of them.

The data in table 1 show that the percentages of colloids, unfree water, and moisture equivalent vary widely for the different soils, all three tending to be high with fine-textured soils and low with coarse-textured soils. When these results are reduced to a ratio basis by dividing the unfree water by the colloids, the moisture equivalent by the unfree water, and the moisture equivalent by the colloids, their significance is revealed. Then it is at once seen that all the different soils have a close relationship within each of the three different ratios; and when it is considered that these different soils vary greatly in their chemical composition and in some of their physical chhracteristics, these ratios are remarkably close. It will be seen that most of the soils in all three cases give a ratio close to the average. The soils the ratios of which seem to vary the most from the averages, are those containing organic matter, as exemplified by Fargo clay loam, Minnesota Clyde silt loam, and Michigan silt loam. One reason that organic soils give a higher ratio is probably because some of the organic matter is almost impossible to disperse and consequently the hydrometer

method may not determine the entire colloidal content (3). Also, since organic matter has a lower specific gravity, and the hydrometer has been calibrated on average loam soil suspension, a smaller amount of suspended material will be shown than there actually is.

At this juncture, it must be also stated that some abnormal soils do not give as close ratios as the ordinary or normal soils shown in table 1. By "abnormal soils" is meant calcareous soils that may contain 50 per cent or more of carbonates; certain subsoils such as the Susquehanna clay C horizon, which seem to be composed mostly of Fuller's earth; mucks and peats; and undecomposed

TABLE 1

Total soil colloids, unfree water, and moisture equivalent and the relationship that exists between any two of them in the various types of soil

SOILS	COLLOIDS	UNFREE WATER	MOISTURE EQUIVALENT	RATIO U. W.* / COLLOIDS	RATIO M. E.† / U. W.	RATIO M. E. / COLLOIDS
	per cent	*per cent*	*per cent*			
Ontario silt loam B	65.0	16.5	38.00	0.254	2.303	0.5843
Fargo clay	62.0	20.0	49.00	0.322	2.450	0.7900‡
Cecil clay loam B	60.7	15.9	38.40	0.262	2.415	0.6328
Ontario silt loam A	58.5	15.2	36.50	0.257	2.402	0.6240
Miami silt loam	54.5	13.6	32.10	0.250	2.360	0.5890
(Ia.) Bremer clay	53.6	13.5	33.20	0.252	2.460	0.6194
Napanee clay loam A	47.4	12.4	31.10	0.262	2.508	0.6560
(Minn.) Clyde silt loam	43.0	12.2	33.20	0.284	2.721	0.7720‡
Manor schist loam B	32.5	9.3	19.80	0.286	2.130	0.6093
Brookston clay loam A_2	30.0	8.9	18.60	0.297	2.090	0.6200
(Mich.) Silt loam	26.0	7.6	15.50	0.292	2.040	0.5960
(Mich.) Silt loam	22.4	7.2	15.90	0.321	2.209	0.7100‡
Fox loam B_2	21.4	7.1	14.85	0.322	2.092	0.6940
Strong's sandy loam	10.4	3.2	6.50	0.308	2.032	0.6250
Average				0.2835	2.301	0.6515

* U. W. = unfree water.

† M. E = moisture equivalent.

‡ Soils containing high content of organic matter.

organic matter in general. For such abnormal soils, probably the best method to use for determining their total colloidal content is the indirect method of the moisture equivalent (6). By determining the moisture equivalent of these soils by the procedure already described their total colloidal content can be ascertained by dividing the percentage of moisture equivalent by the average ratio of moisture equivalent.

When this procedure is applied to Fargo clay and Clyde silt loam in table 1, it is found that the colloidal content of these soils is considerably increased, and their ratio is thereby brought much closer to that of the inorganic soils.

It must be remembered, however, that the difference in the specific gravity between the inorganic and organic soils, introduces many complexities which must be taken into consideration in any comparative relationship studies.

Aside from these abnormal or special cases, the results in table 1 tend to show quite conclusively that the material which the hydrometer method determines at the end of 15 minutes and designates as colloids, practically controls the physical characteristics of soils, at least those that deal with moisture relationships. If this were not the case, the ratios would not be so close for the various types of soil.

These ratios reveal one more significant thing, namely, the hydrometer for determining colloids, the dilatometer for determining unfree water, and the vacuum pressure method for determining moisture equivalent of soils, would seem to be fundamentally sound and correct, at least they tend to give true comparative results for the various soils.

Indirect determinations

Since the relationships between colloids and unfree water, between unfree water and moisture equivalent, and between colloids and moisture equivalent are so close, when one result is known the other can be calculated from the respective average, as indicated by the formulas:

$$\text{Colloids} \times \frac{\text{Moisture equivalent}}{\text{Colloids}}\ \text{ratio} = \text{per cent moisture equivalent},$$

$$\frac{\text{Moisture equivalent}}{\frac{\text{Moisture equivalent}}{\text{Colloids}}\ \text{ratio}} = \text{per cent colloids},$$

$$\text{Colloids} \times \frac{\text{Unfree water}}{\text{Colloids}}\ \text{ratio} = \text{per cent unfree water},$$

$$\frac{\text{Unfree water}}{\frac{\text{Unfree water}}{\text{Colloids}}\ \text{ratio}} = \text{per cent colloids},$$

$$\frac{\text{Moisture equivalent}}{\frac{\text{Moisture equivalent}}{\text{Unfree water}}\ \text{ratio}} = \text{per cent unfree water}.$$

Wilting coefficient

The unfree water shown in table 1, has a special interest and significance in that it might represent the wilting coefficient of plants, and in that event, the latter can be determined indirectly very rapidly and simply by the hydrometer method.

The belief that the unfree water of the various soils as obtained by supercooling and freezing them at the temperature of −0.5°C. might represent their respective wilting coefficient points, is based upon two well-founded facts: First, the careful researches of Shull (8) on the measurement of the surface forces of soils, seem to show that the suction force of the soil for water at the wilting point is about 4 atmospheres; second, according to the laws of the freezing point depression the suction force of soil for water at the freezing point depression of −0.5°C. is about 6 atmospheres. This latter result is obtained from the well-known facts that a molar solution has an osmotic pressure of about 22.4 atmospheres and that the freezing point depression of a water solution containing 1 gram molecule per liter of non-ionized salt is 1.86°C. According to this figure, and assuming the relationship is linear, the suction forces of soils for water corresponding to various freezing point depressions are about 5.95 atmospheres for −0.5°C., 17.9 atmospheres for −1.5°, 29.8 atmospheres for −2.5°, 47.7 atmospheres for −4°, and 937 atmospheres for −78°.

If the unfree water then as obtained at the temperature of −0.5°C. does actually represent the wilting coefficient points of soils, then the wilting coefficient of practically any soil can be determined very simply in only 15 minutes by means of the hydrometer method. The formula that may be used to accomplish this indirectly is as follows:

$$\text{Percentage colloids} \times 0.2835 = \text{wilting coefficient},$$

Since the results obtained by the foregoing formulas have not been checked as yet by actual experimentation with plants, it cannot be said how correct they are. Judging, however, from the apparent soundness of the principles on which they are based, and also from a comparison of magnitude of actual wilting coefficient data, as obtained by Briggs and Shantz (7) on somewhat similar soils, it would appear that they might be quite correct, at least in the normal soils.

It is thus seen then, that on account of the great rapidity, distinct simplicity of manipulation, and reasonable degree of accuracy, the hydrometer method may not only be employed to estimate the colloidal content of soils very rapidly, but may also be used to determine very quickly several characteristics of soils. These indirect determinations, however, should be regarded only as approximately correct.

SUMMARY

Experimental results are presented which tend to show that the soil material which the hydrometer method determines at the end of 15 minutes and designates as total colloids of the soil, has a close relationship to the moisture equivalent and unfree water of soils.

On account of this close relationship, these physical characteristics of soils may be indirectly determined very quickly by the hydrometer method.

It also appears that the wilting coefficient of soils also may be indirectly determined by the hydrometer method very rapidly.

These various indirect determinations should be regarded only as approximately correct.

REFERENCES

(1) Bouyoucos, G. J. 1917. Classification and measurement of the different forms of water in the soil by means of the dilatometer method. Mich. Agr. Exp. Sta. Tech. Bul. 36.

(2) Bouyoucos, G. J. 1925. The heat of wetting as a new means of estimating the colloidal material on soils. *Soil Sci.* 19: 153–162.

(3) Bouyoucos, G. J. 1927. The hydrometer as a new and rapid method for determining the colloidal content of soils. *Soil Sci.* 23: 319–330.

(4) Bouyoucos, G. J. 1928. Making mechanical analysis of soils in fifteen minutes. *Soil Sci.* 25: 473–480.

(5) Bouyoucos, G. J. 1928. The hydrometer method for making a very detailed mechanical analysis of soils. *Soil Sci.* 26: 233–238.

(6) Bouyoucos, G. J. 1929. A new, simple, and rapid method for determining the moisture equivalent of soils, and the rate of soil colloids on this moisture equivalent. *Soil Sci.* 27: 233–240.

(7) Briggs, L. J., and Shantz, H. L. 1912. The wilting coefficient for different plants and its indirect determination. U. S. Dept. Agr. Bur. Plant Indus. Bul. 230: 1–83.

(8) Shull, C. A. 1916. Measurment of the surface forces in soils. *Bot. Gaz.* 62: 1–31.

LOCAL VARIATION OF SOIL ACIDITY IN RELATION TO SOYBEAN INOCULATION

GEORGE Z. DOOLAS[1]

University of Missouri

Received for publication February 24, 1930

Irrespective of the extensive investigation on the injurious effects of soil acidity on inoculation and growth of legumes, the nature of these effects is not yet fully understood. The question naturally arises: is the failure of nodulation due to the detrimental effects of the hydrogen-ion or hydroxyl-ion concentration directly on the legume bacteria or does the concentration in some way bring about unfavorable conditions in the plant which prevent the entrance of the specific bacterium or prevent nodule formation even if the bacterium is present in the plant tissue. Since this question has an important bearing on the problem of obtaining good legume inoculation in certain acidic soils, the experiments reported herein were conducted to determine whether successful inoculation might be obtained by reducing the acidity in only a portion of the soil; and in the meantime to study the effects of different hydrogen-ion concentrations in scattered local areas in the same soil on nodulation, root growth, and top growth of soybeans.

LITERATURE REVIEW

Fred and Davenport (7) found that the favorable reaction range of *Bac. radicicola* is closely correlated with that for its respective host. As a consequence of their findings, they were enabled to classify *Bac. radicicola* from different plants according to pH limits for growth or reproduction. For the soybean organism the critical acidity was found to be at pH 3.3. Bryan (4) observed that the limits for inoculation of soybeans were pH 4.6 and 8.0, whereas those for the growth of the soybean plant were pH 3.8 and 9.6. The aforementioned data suggest that if the lack of nodulation is ascribable to some failure of the organism, certainly it is not because the organism is not present, since it will live in a hydrogen-ion concentration (pH 3.3) far beyond the limits (pH 4.6) in which it will inoculate. However, it is possible that it loses its power of motility or virulence to enter into the plant root tissues, or if it enters, it may fail to perform its normal function.

Sniezko (17) more recently found that either too high or too low pH induced the production of the vacuolated form of legume bacteria, which is thought by Bewley and Hutchison (3) and by Thornton and Gangulee (18) to lack the power to inoculate. According to Thornton and Gangulee, milk and calcium phosphate in the inoculum suspensions favor the production

[1] Thanks are due to Dr. Wm. A. Albrecht, under whom the author worked, for his valuable criticisms and suggestions in connection with the present work and the preparation of this paper.

of the supposedly virulent forms; and on this basis they recommend the inoculation of the soil with bacterial suspension in milk to which is added 0.1 per cent of calcium phosphate. They attribute the beneficial effects to the phosphorus.

Wilson (21) Perkins (14), and Kellerman and Robinson (10), have found the calcium compounds, with the possible exception of the sulfate and nitrate, effective in stimulating nodule formation in sand and soil cultures under greenhouse conditions.

Scanlan (16) and Albrecht and Davis (1) obtained increases both in nodulation and growth of legumes on several Missouri acid, and supposedly calcium-deficient, soils to which either basic or neutral salts have been added both under greenhouse and field conditions.

Bryan (4) observed that outside of certain limits of the hydrogen-ion concentration of the growing medium in which nodulation was depressed, the roots were discolored and injured, whereas the secondary roots were stubby.

Whitson and Chapman (20) report that in many soil types in Wisconsin, acidity is not the only factor limiting growth but that deficiency of calcium, of potassium, or of phosphorus may also be a factor.

Since acidity in soils is generally associated with low amounts of calcium, and since legumes utilize comparatively large amounts of this element, it is difficult to ascertain whether the limiting factor is the reaction or the deficiency of calcium. True (19) has pointed out that calcium is the most important single element, especially during the early stage of plant growth, since it plays a unique rôle in the middle lamellae of the plant and in absorbing mechanism of the plant root. He found that the magnitude of the limiting quantity of calcium and its absorbability from dilute concentrations of calcium in solution are very closely correlated with the degree of acidity tolerance of plants. The legumes were found to be among those plants which normally require comparatively large amounts of calcium for normal growth to maturity.

Arrhenius (2) carried out extensive researches on the influence of the different forms of acidity, such as free, exchangeable, and hydrolytic, on plant growth and on the absorption of the nutrients. Mevius (13) quotes some data from Arrhenius on the absorption of the nutrients at different pH values, and states that the absorption is proportional to the pH; however, the permeability of the cell does not depend on the H or OH ion alone, but also on the kind, number, and mutual relationships of the other ions. Bryan (5) observed a similar decrease of calcium content in alfalfa, alsike clover, and red clover with an increase of acidity of the water cultures in which they were grown. Kirste (11) reports that the unfavorable influence of the exchangeable acidity could be removed by treatment of some of the more susceptible crops such as barley, beets, and mustard, not only with calcium carbonate but also with soda.

Jansen (8), working with some southern legumes, obtained increased growth and nodulation by modifying the acidity by addition of sodium hydroxide as well as of calcium hydroxide. Karraker (9) grew alfalfa in pots with part of the roots in limed and part in unlimed acid soils and concluded from the results obtained that the effect of soil reaction upon nodule formation must be one of localized character in the plant, or a direct effect of soil hydrogen-ion concentration on the bacteria. Albrecht and Davis (1) grew soybeans with a part of the roots grown in an unlimed portion and part in a limed portion of an acid soil. From the results of this and other experiments, they concluded that calcium is beneficial and that its effect on nodulation is local in character.

McCool (12) observed that local application of small amounts of lime drilled with the seed resulted in very good increases of alfalfa crop in many acid soils in Michigan.

The studies mentioned in the review reveal two interesting points. The first suggests that other factors, particularly calcium deficiency, may influence the effects of soil acidity on inoculation. The second leaves the impression that soil acidity, and calcium as well, may affect legume inoculation through (*a*) their influences on the plant and (*b*) their influences on the bacteria. For fuller knowledge of how soil acidity injures inoculation it is necesary, therefore, (*a*) that the calcium deficiency factor be eliminated, and (*b*) that a differentiation

be made between the plant and the organism, as each may be influenced by acidity in respect to inoculation. Accordingly, in the following experiments whereby it is intended to study the effect of local variation of hydrogen-ion concentration on inoculation, an attempt was made to eliminate the factor of calcium deficiency, and thus attempt to measure only the effects of the hydrogen-ion concentration on inoculation as they may be wrought through the plant and the organism separately.

EXPERIMENTAL

The soil used in these experiments was surface Putnam silt loam from a field in which inoculation of soybeans was observed to be difficult. It is of medium fertility and has a reaction of pH 4.0 to 5.5. Its electrodialyzable calcium content is only 4 m. e. to 100 gm. of soil. The titration curves, as

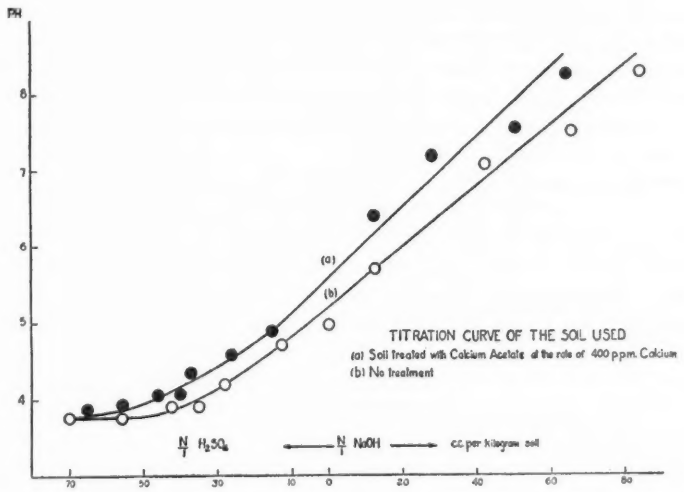


FIG. 1. TITRATION CURVE OF THE SOIL USED

determined from the reaction changes after equilibrium had been reached, are presented in figure 1. When calcium acetate was added a larger quantity of acid and a smaller amount of base were required to obtain the same pH than when no calcium acetate was added.

Soybeans were grown under greenhouse conditions in four series of pots, designated as A, B, C, and D. Series A and B were prepared specially for the study of the effects of local variation of the hydrogen-ion concentration. An attempt was made in these to separate the influences of the hydrogen-ion concentration on inoculation as they may be effected (*a*) through the plant and (*b*) through the organism. This was deemed possible by growing the roots of the plant through different soil areas varying in reaction. In these pots the soil was divided into two zones by means of paraffin screen pots. These

pots were made by dipping pot-shaped, screen wire frames into a molten mixture of 4 parts paraffin and 1 part petrolatum. This material when solidified, allows the penetration of the roots without permitting any leakage at the points of root penetration. Into each of these paraffin pots 400 gm. of soil was placed. They were buried, almost to their tops, in 2,500 gm. of soil contained in gallon jars. After the treatments and after the air-dried soil was mixed in the ball mill, the pots with moistened soil were allowed to stand for about 30 days in order that an equilibrium might be reached. The soil both in the paraffin pots and in the jars was inoculated.

Presumably, therefore, this system contained both the bacteria and the plant roots in two separate zones which differed in reaction and calcium content. Such conditions permitted the plant at least to draw its nutrients from the zone of favorable reaction. If acidity is only local in its effecct on inoculation, nodules would be formed, at least under these conditions, on the roots in the zone of the favorable reaction. Conversely if it is general, or systemic, through its effects on the plant, then irrespective of the presence of the favorable soil environment for the bacteria no nodules would be likely to form in any part of the roots.

Series D was prepared in order to ascertain whether the calcium as supplied by the calcium acetate additions would modify to any perceptible extent the influence of acidity on inoculation. Series C and D, added as a means of control for series A and B, consisted of 1-gallon jars each containing 3,000 gm. of soil. The reaction in these was changed in the same order as in the series A and B. In addition, series C and D included pots in duplicate of pH 7.0, 7.3 to 7.5, and 8.2 to 8.35. These ranges were effected by the addition of a dilute solution of sodium hydroxide. To pots of series D, calcium acetate was added in an amount equivalent to 2 m. e. of calcium to 100 gm. of soil, corresponding to that added to series A and B..

Both the soil and seed were inoculated with artificial cultures. Five soybean plants were allowed to grow in each pot for 80 days. They were irrigated with distilled water, added on the surfaces in the case of the paraffin pots and through a small flower pot sunk in the soil.

RESULTS

Nodule formation was good and of about equal extent in all jars of series A, or in those soil zones (pH 5.6) outside of the paraffin pots, except in one case in which the reaction in the paraffin pot was initially brought to a pH as low as 3.8. In this case not only were nodules absent, both inside and outside of the paraffin pot, but even root growth was very poor, being badly injured within, and very sparse without, the paraffin pot. The failure of nodule formation exterior to this most acid inner zone appears to have been the result of nutritional disturbances brought about probably through injury to the inner roots. These results are evident from the data in column 4, series A, table 1, and are shown clearly in the upper halves of figure 1 and 2 of plate 1. As noted from

the nodule counts in column 5, series A, table 1, nodulation was absent inside the paraffin pots of initial acidities ranging from pH 3.8 to 4.6, but was present in those of initial acidities of pH 4.6 and pH 5.0

TABLE 1

Effect of local variation in soil reaction on the nodulation and growth of soybeans

(Series A and B)

SULFURIC ACID ADDED*	pH OF SOIL		NODULES PER POT (AVERAGE)		WEIGHT PER POT (AVERAGE)		CONDITION OF GROWTH
	Planting	Harvest					
cc. Inside†	Inside	Inside	Outside	Inside	Tops	Roots	
Series A							
0	5.05	5.15	10	20	1.75	0.38	Roots healthy, inside and outside
0.72	4.65	5.0	6	7	1.65	0.31	
1.75	4.35	4.6	9	0	1.64	0.32	Out, good; inside, brown, acid-injured
3.20	4.175	4.2	12	0	1.70	0.27	Out, fairly good. Inside, no secondary roots
3.57	3.9	4.1	20	0	1.50	0.38	Primary, acid-injured
4.7	3.8	4.0	0	0	1.15	0.15	Out, few roots. Inside badly injured
Series B							
Outside‡	Out side	Outside	Inside	Outside	Tops	Roots	
0	5.0	5.0	37	0	1.87	0.35	Good, inside and outside
0.72	4.6	4.85	23	0.2	2.09	0.29	
1.75	4.3	4.6	34	0	1.90	0.40	Inside good. Out, few secondary roots, all black and acid-injured
3.20	4.0	4.2	32	0	1.34	0.33	
3.50	3.95	4.0	30	0	1.36	0.30	Inside, good. Out, few or none
4.50	3.875	4.0	1–29	0	1.76	0.39	Inside, good. Out, none

* Normal sulfuric acid per 100 gm. soil.

† Term "inside" refers to soil within the screen paraffin pot given the sulfuric acid. The soil outside of the screen pot received calcium acetate equivalent to calcium at 400 p.p.m. of soil, giving it a pH of 5.6 to 5.8 constant throughout the growth period.

‡ The term "outside" refers to the soil exterior to the screen paraffin pot. Soil treatments were the same as* but reversed with reference to location inside or outside the paraffin pot.

In the case of series B, in which the acid and more favorable reaction zones were arranged in a reverse order to that in series A, the effects on nodulation and root growth were also correspondingly reversed. Accordingly, nodules were absent in the jars or in the zones outside of the paraffin pots of pH 3.8 to 5.0 except in one of the duplicate pots of pH 4.6, but were abundant inside

of the paraffin pots or the zones of pH 5.6 (columns 4 and 5, series B, table 1 and lower halves of figures 1 and 2 of plate 1).

The data reveal a correlation between increase of acidity and increase of injury to the roots below pH 4.6. Beyond this limit they were black and rather tough, the primary roots were fewer, short, and stubby. In general, root growth decreased with increase of acidity within its zone.

TABLE 2

Nodulation and growth of soybeans when the entire root system grew in soil uniform in reaction

(Series C and D—spring planting)

POT TREATMENT	pH	NUMBERS PER POT		WEIGHT PER POT		CONDITIONS OF GROWTH
		Plants	Nodules	Tops	Roots	
				gm.	*gm.*	
*C	3.80	0	0, 0	0	0	Cotyledons appeared but no leaves were formed
†D		0	0, 0	0	0	
C	3.85–3.95	5	1, 0, 0, 0	1.11	0.13	Roots and tops were injured; leaves dried at margins and tips, and most of them died
D		5	7, 1, 0, 0	1.15	0.15	
C	4.10–4.25	5	0, 0, 0, 0	1.02	0.13	
D		5	2, 0, 0, 0	1.20	0.11	
C	4.35	5	0, 0	1.77	0.20	Tops injured. Roots black
D		5	0, 0	1.53	0.16	
C	4.65	5	0, 0	1.39	0.17	Tops and roots moderately injured
D		5	0, 0	1.25	0.13	
C	5.0	5	5, 3	1.91	0.22	Roots healthy. Tops, slightly injured
D		5	1, 0	1.30	0.15	
C	7.1–7.2	5	0, 13	2.00	0.12	Soil puddled and too wet. Tops good. Roots short but healthy
D		5	0, 16	3.10	0.16	
C	7.3–7.5	5	0, 0	0.50	0.06	
D		5	0, 1	1.30	0.06	
C	8.2–8.4	0				Soil too wet. Sparse root growth
D		3, 0	0, 0	0.475	0.07	

* The physical condition of the soil was poor in the non-calcium alkaline pots, but it was better in the calcium-treated pots.

† D—400 p.p.m. calcium added per pot.

As one might expect, the magnitude of disturbance of nodule formation was more or less parallel with the degree of root injury. Thus, nodule formation was either depressed or totally absent in the root segments that were damaged, whereas it was normal in the root segments that were healthy. However, the extent to which nodule formation took place on an apparently equally

damaged root segment appears to have varied according to some not well understood factors, probably such as nutrition distrubances that might have been caused through injury to other parts of the plant. Nodule formation, therefore, tended to vary, in general, according to the healthiness of the local root tissue and according to the nutritive supply to it.

There were greater numbers of nodules in the inner than in the outer zones of the same pH. A critical study of nodule counts given in column 5, table 1, suggest that the critical reaction limit for nodule formation occured at a higher pH for the roots in the outer zone, or more distant portion of the roots, than for the roots closer to the base of the stem which were grown in the inner zone. Both nodule numbers and root growth decreased according to the decrease of pH in the soil area, but remained realtively constant in the zones of constant pH (5.6). Figure 1 of plate 1 and the data in columns 6 and 7, table 1, show that the depressing effects of acidity were less on the growth of tops, than upon the nodulation, and upon root growth.

No definite correlation was observed to exist between the amount of top growth and the relative abundance of nodules.

In both series C and D, in which the entire medium had the same reaction, nodule formation and growth of both tops and roots were markedly subnormal and decidedly inferior to those of the pots of the corresponding reaction in series A and B, as shown in plate 2 and table 2. Nodulation occurred rather irregularly. For example, few nodules were found on some plants grown in soil of reaction pH 4.0, whereas none was found in some pots of reaction higher than pH 4.0. It is interesting to note that nodules occurred very frequently in that portion of the roots lying just below the small pot through which irrigation water was applied. Incidentally this circumstance suggests the possibility that inoculation was influenced by the local environment as it might have affected the root segment directly.

Unlike series A and B, the tops in the pots of series C and D exhibited marked irregularities, which increased with decrease in pH. The leaves were curled, beginning first at the tips, which were burned and folded inward. They were dried at the margins but remained green toward the midrib.

There were no perceptible differences with respect to nodule formation and root and top growth between the pots with and without calcium additions, except in the alkaline ranges of 7.0 to 8.3 wherein calcium might have been beneficial. Calcium might have reduced the bad physical condition of the soil by counteracting the deflocculating effects of the added sodium hydroxide, or it might have been a source of soluble calcium to overcome the possible decreased solubility of the soil supply. Since calcium was apparently not a factor in inoculation or plant growth in this case, the influence of the hydrogen ion seems to be responsible for the nodulation irregularities.

The behavior of roots toward acidity was the same as those in the corresponding acid zones of the divided pots, series A and B. The character of the injury exhibited on roots was similar to that observed by Bryan (4). In many re-

spects the irregularities observed on the plants in general, and particularly on the tops, seems to have been very similar to those observed generally in soybeans grown in this greenhouse in calcium-deficient media.

The extent to which acidity inhibits or prohibits inoculation, appears, according to general observation in these trials, to be influenced to some extent

TABLE 3

Nodulation and growth of soybeans when the entire root system grew in soil of uniform reaction

(Series C and D—Summer Planting)

POT TREATMENT	pH	NODULES PER POT (AVERAGE)	WEIGHT PER POT		CONDITION OF GROWTH
			Tops	Roots	
			gm.	*gm.*	
C	3.75–4.00	0	2.00	0.25	
D*		0	1.50	0.15	
C	4.15–4.20	1	2.30	0.17	Leaf and root injury increased with decrease in pH. Few short and stubby secondary roots
D		2	1.82	0.18	
C	4.25–4.30	2½	2.2	0.24	
D		0	1.9	0.18	
C	4.40–4.50	14	3.23	0.30	Crown roots were brown or black; leaves were injured
D		2	2.90	0.19	
C	4.55–4.65	12	3.15	0.39	
D		7	2.50	0.19	Roots and tops healthy but crowns were brown
C	4.90–5.00	17	2.90	0.38	
D		4	2.55	0.34	
C	6.0	30	3.6	0.38	
D		20	3.0	0.30	
C†	7.0	22	3.7	0.25	
D		35	3.4	0.30	Both roots and tops healthy; leaves deeper green than in the above
C	8.3	4	3.0	0.20	
D		18	2.8	0.25	
D	8.70	0	2.30	0.18	

* D = 400 p.p.m. calcium added per pot.

† The soil in the alkaline pots was diluted with acid-extracted quartz sand.

by the weather factors, particularly light, which is difficult to control. Accordingly, the actual lower pH limit is not necessarily always a fixed value. When the pots of series C and D (table 3), were replanted in August, 6 months after the first planting, nodule formation was greater and growth of tops and roots was generally less injured; and nodulalation took place at a lower pH,

than in the first planting, which took place in the winter and spring months. These observations suggest that the extent to which acidity becomes injurious to inoculation is also dependent on the external factors, which may influence the growth of the host plant.

It is evident from the reported observations that the harmful effects of acidity on nodulation and root growth were, in the pH ranges employed here, namely pH 4.0 to 5.6, more or less local in character. The extent to which acidity injured other parts of the plant outside of the acid zones, appears to have been determined by the extent to which the nutritive supply to those

TABLE 4

Inoculability of soybean organism from pots of varying reaction

(Test made in sterile sand culture pots)

SOURCE OF INOCULATING MATERIAL		AVERAGE NUMBER OF NODULES PER PLANT IN THE SAND CULTURE POTS
Pot	pH in the pot	
Commercial culture	....	6.0
A—inside	3.8	16.0
B—outside	3.8	0.7
C	3.8	13.0
D	3.8	8.0
A—outside	5.6	16.5
C	4.0	12.0
D	4.0	19.0
A—inside	4.65	14.0
B—outside	4.65	12.0
A—inside	5.0	10.0
C	5.0	12.0
C	7.0	4.0
D	7.0	13.0

other parts was disturbed. The very sparse growth of roots in the outer soil area exterior to the most acidic inner zone (pH 3.8), and the abnormalities of the tops in the pots (pH 3.8 to 5.0) in series C and D, as compared with the absence of such abnormalities in the paraffin pots of series A and B, suggest such a case of nutrition disturbance.

Bac. radicicola remains viable in acid soils

In order to ascertain more definitely whether depression or failure of nodule formation in these experiments was due to death or to loss of viability of the organism in these acid media, the following test was made. Four months

after the planting of the soybeans in the pots of the preceding experiments, a 2-gm. sample of soil was collected from each of the several pots representing different acid intensities. This sample was used to inoculate soybean seeds which had been planted in sand culture pots. Sterile control pots were planted with these inoculated seeds. The test was made with the utmost care in order to avoid contamination by soybean bacteria from other sources.

Table 4 reveals that in these sand cultures viable bacteria had been transferred from every single soil pot of the previous experiment, including those with the extreme acidity of pH 3.75, in which no nodules originally occurred. The nodule in these sand cultures were uniformly plentiful, save in one case of the sample of soil (pH 3.8) outside of the paraffin pot series B. The nodule numbers in this case were markedly lower than in the other pots. This apparent depression of either the viability or inoculability of *Bac. radicicola* can not be attributed to the effects of strong acidity alone, for the reason that the viability of the organism was found to be just as good in the pots of the same pH of the other series. It must be remarked that these were the acid zones (pH 3.8) outside of the paraffin pots or jars, in which hardly any roots were grown. It may be possible, therefore, that the viability of the organism is favorably influenced by its association with the living roots of its respective plant host.

If the depression or prevention of nodule formation occurred because of the effects of acidity on the legume bacteria, such disturbances would result (*a*) if acidity prohibited entrance of the bacteria into the root, (*b*) if the reaction of the tissue disturbed the bacterial function of nodule production, or (*c*) if acidic conditions interfered with the nutritive supply to the organism and nodule tissue. Failure of the organism to enter the roots cannot be due to the death of the bacteria in the acid ranges used. Unfortunately no measurement of acidity of the root tissue was made. Since the roots were badly injured in the acid zones, the disturbance in the nutritive supply to the root area of nodule formation seems a plausible explanation.

SUMMARY

When soybeans were grown in the greenhouse with the roots extending partly through acid soil zones of pH 3.8 to 5.0 and pH 5.6, depression of nodulation and root injury occurred on the segments within the media with pH ranging from 3.8 to 4.6. In the soil area with pH 5.6 the root segments were healthy and, with but few exceptions, uniform in nodulation.

The paraffined screen pot permitted growing different portions of the same root in areas of different reactions within the same pot.

Apparently healthy plants of uniform growth resulted when part of the root system was partially injured in the extremely acidic soil areas (pH 4.0 to 5.6), and partially healthy plants in the less acidic media (pH 5.6). When the entire root system was surrounded by the extremely acidic soil, however, abnormalities in the growth both of roots and tops were evident.

It seems probable that depression of nodulation by acidity above the acid degree of pH 3.8 or 4.0 occurred through the action of the acidity on the plant tissue locally, or through the disturbance of the nutritive supply by the plant to the organism and tissue area in question.

Nodule organisms retained their viability in all soil media with degrees of acidity ranging from pH 3.8 to 8.3.

REFERENCES

(1) ALBRECHT, W. A., AND DAVIS, F. L. 1929 Relation of calcium to nodulation of soybeans on acid and neutral soils. *Soil Sci.* 28: 261–274.

(2) ARRHENIUS, O. 1925 Der Kalk bedarf des Bodens von Pflanzen physiologischen Standpunkt: II. Die Bodenreaklion und das Wachstum der hoheren Pflanzen. *Ztschr. Pflanzenernänr. u. Düngung.* (A) 4: 30–52.

(3) BEWLEY, W. F., AND HUTCHISON, H. B. 1920 On the changes through which the nodule organism (*Ps. radicicola*) passes under cultural conditions. *Jour. Agr. Sci.* 10:144–182.

(4) BRYAN, O. C. 1922 Effect of different reactions on the growth and nodule formation of soybeans. *Soil Sci.* 13: 271–285.

(5) BRYAN, O. C. 1923 Effect of reaction on growth, nodule formation, and calcium content of alfalfa, alsike clover, and red clover. *Soil Sci.* 15: 23–35.

(6) BRYAN, O. C. 1923 Effect of acid soils on nodule-forming bacteria. *Soil Sci.* 15: 37–40.

(7) FRED, E. B., AND DAVENPORT, A. 1919 Influence of reaction on nitrogen-assimilating bacteria. *Jour. Agr. Res.* 14: 317–333.

(8) JANSEN, G. 1929 The comparative acid tolerance of some southern legumes. *Soil Sci.* 27: 469.

(9) KARRAKER, P. E. 1927 Production of nodules on different parts of the root system of alfalfa, growing in soils of different reactions. *Soil Sci.* 24: 103–109.

(10) KELLERMAN, K. F., AND ROBINSON, T. R. 1906 Conditions affecting legume inoculation. U. S. Dept. Agr. Bur. Plant Indus. Bul. 100.

(11) KIRSTE, H. 1925 Ueber das Pflanzwnwachstum auf sauren Boden. *Ztschr. Pflanzenernähr. u Düngybg.* (A) 5: 129–194.

(12) MCCOOL, M. M. 1927 Methods of applying lime. *Jour. Amer. Soc. Agron.* 19: 198–199.

(13) MEVIUS, W. 1926 Die direkte Beeinflussung der pflanzen Zelle durch die Wisserstuffionen konzentration des Nuhrsubstrates. *Ztschr. Planzenernähr, u, Düngung.* (A) 6: 89–98.

(14) PERKINS, A. T. 1924 The effect of several mineral fertilizers upon the nodulation of Virginia soybeans. *Soil Sci.* 17: 439–447.

(15) PERKINS, A. T. 1924 A note on the nodulation of soybeans. *Soil Sci.* 17: 449–456.

(16) SCANLAN, R. W. 1928 Calcium as a factor in soybean inoculation. *Soil Sci.* 25: 313–324.

(17) SNIEZKO, S. 1928 Extrait du bulletin de l'Academie Polonaise des Sciences Mathematiques et Naturelles. Serie B: Sciences Naturelles (Botanique).

(18) THORNTON, H. G., AND GANGULEE, N. 1926 The life of the nodule organism *Bac. radicicola* (Beij) in soil and its relation to the infection of the host plant. *Proc. Roy. Soc. (London) Ser. B.* 99: 427–451.

(19) TRUE, R. H. 1921 The function of calcium in the nutrition of seedlings. *Jour Amer. Soc. Agron.* 13: 91–107.

(20) WHITSON, A. R., AND CHAPMAN, H. D. 1927 Soil and fertilizer experiments at the Wisconsin Experiment Station. *Wis. Agr. Exp. Sta. Bul.* 59: 19.

(21) WILSON, J. K. 1917 Physiological studies of *Bacillus radicicola* of the soybean and of factors influencing nodule formation. N. Y. (Cornell) Agr. Exp. Sta. Bul. 386.

PLATE 1

Effect of Varying Degrees of Acidity as Soybean Growth

Fig. 1. Plant growth and root development exterior to paraffin pots with soils of varying degrees of acidity.

Above: Reaction outside constant (pH 5.6); varied inside (pH 5.0 to 3.8).

Below: Reaction outside varied (pH 5.0 to 3.8); constant inside (pH 5.6).

Fig. 2. Root development of soybeans as affected by soils of different degrees of acidity within paraffin pots.

Above: Varying degrees of acidity in soil of plant crown area (pH 5.0 to 3.8) and constant acidity in remaining root zone (pH 5.6).

Below: Constant acidity in soil of plant crown area (pH 5.6) and varying degrees of acidity in remaining root area (pH 5.0 to 3.8).

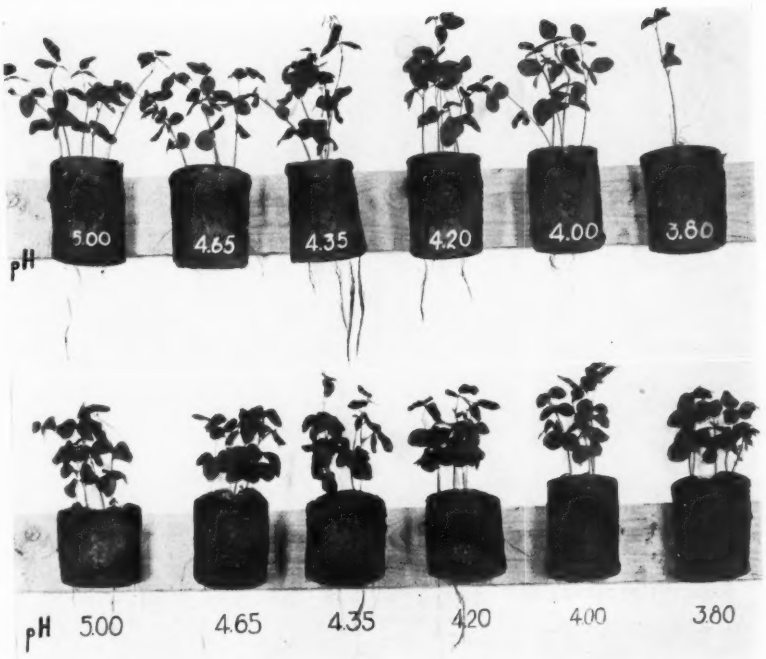

pH
5.00
4.65
4.35
4.20
4.00
3.80
pH 5.00 4.65 4.35 4.20 4.00 3.80

FIG. 1

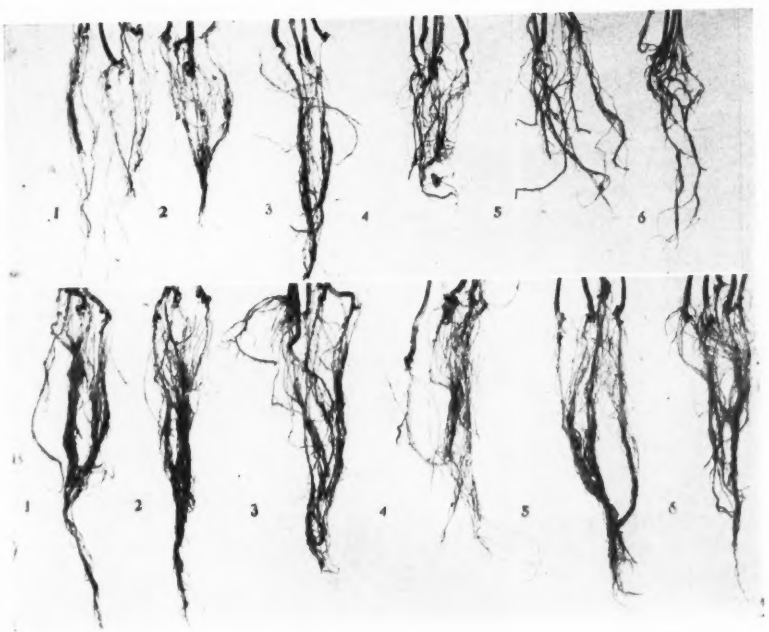

1
2
3
4
5
6
1
2
3
4
5
6

FIG. 2

PLATE 2

SOYBEAN GROWTH IN CONSEQUENCE OF VARYING DEGREES OF ACIDITY IN THE SOIL

Above: Calcium acetate added, 400 p.p.m. Ca to each pot.
Reaction: Left to right, pH 3.75, 3.90, 4.05, 4.20, 4.35, 4.60, 5.0, 7.1, 7.3, and 8.25.

SEASONAL VARIATION IN THE NUMBER OF TWO SPECIES OF RHIZOBIUM IN SOIL

J. K. WILSON

Cornell University

Received for publication February 28, 1930

The growth and longevity of bacteria in soil are dependent upon climatic factors, the physical and chemical make-up of the soil, the composition of the soil air, the temperature, the time of year, the associative action of the edaphon contained in the soil. Since bacteria respond to their environment it would be expected that as any one or more of these factors vary the bacterial content would also change. Sometimes these factors operate to increase and sometimes to decrease the number of bacteria that may be found at any season. Studies have been made of the fluctuations which occur in bacterial number from one season of the year to another season, but they have been made of the heterogeneous flora that will develop on a certain type or types of media.

Francé (4) reports a minimum of the heterogeneous soil flora in winter with a maximum in spring and fall. Brown and Halverson (1) found from a similar study that during the summer and early fall the bacteria did not develop parallel with either moisture or temperature, and during much of the year other undetermined factors seemed to control bacterial development. It was found also that the soil bacteria decreased in late fall with a drop in temperature until the soil became frozen, when the number rose with decreased temperature and fell with slightly higher temperature regardless of the moisture content. Upon thawing of the soil, however, the numbers of bacteria decreased. With a further rise in temperature which made conditions favorable for growth, an increase in bacteria occurred, which reached a maximum by June 19. Two maximum counts were observed during the year—in February and June. Since these counts were of the heterogeneous flora it is not known whether such fluctuations were due to the destruction of one or more types of bacteria or whether some of every type was killed. Lyon, Bizzell, and Conn (8) found the heterogeneous flora to increase if the soil was well frozen but to decrease decidedly after a thaw. Such an increase was explained by Conn (3) as being due to the liberation of a large number of colonies that would not otherwise be recognized. No satisfactory explanation was offered for the sudden decrease after thawing. Vass (10) tried to explain the rise in counts in frozen soils as being due to the breaking up of clumps or colonies of bacteria and not to growth and multiplication.

Vanderleck (9) suggested, from observations he made, that raw material which is available for bacterial decomposition may be responsible for bacterial increase in January, since there was no increase in soils where raw material was absent, but no such explanation was offered when in frozen soils in March an increase in numbers was encountered amounting to from two to four times the original number and which was followed by a decided decrease when the soil thawed. He also reported that a high soil moisture content counteracted the frost action, whereas a low moisture content aided in the depression of bacterial development, and that a sudden severe frost killed most of the bacteria in exposed soil. Harder (6) presents data

which show a close relation between the moisture content and the number of bacteria in soil, yet a distinct retardation by cold which occurred with a high moisture content, was not explained.

One may conclude from such data that there is a decrease in the heterogeneous bacterial flora of the soil sometime during the winter, which cannot be explained entirely by a deficiency of organic matter, by soil temperature conditions, or by moisture as such. It seems that whatever the factor or factors may be that cause a reduction in number of bacteria they do not bring about a sterilization of the soil, although certain definite types of organisms may be almost entirely eliminated from certain soil types, as has been found by Jones and Murdock (7) and by many others.

In an effort to find to what the protective action in soils and solution culture is due, Vass (10), in a study of the influence of low temperature on Rhizobium, states that the concentration of the medium, the length of time of the exposure, and the degree of cold, are the three important factors that determine the power of resistance of the bacteria to low temperature; that the protective action due to the concentration of the medium seems to be effective only in cases in which the eutectic point of the substances in solution is below the temperature of the exposure; and that the death of the bacterial cell when exposed to low temperature seems to be due to the withdrawal of water from the semipermeable membrane or outer layer of the cell. In this connection the work of Giltner and Langworthy (5) may be mentioned. These authors also worked with Rhizobium and found that the survival of bacteria in desiccated soil is due in part to a retention of moisture in hygroscopic form. The removal of moisture by desiccation is in a measure comparable to the withdrawal of moisture by freezing.

Very few studies have been made of the seasonal variation of definite species of organisms. An indication of what may happen to a certain genus was suggested by some results which were obtained from a study of the legume bacteria population of the soil. In a report of this study (13), among other things, it was pointed out, through literature cited and through data given, that soils may have a wide variation in the number of Rhizobium. The species that produce nodules on Medicago ranged in numbers from none in 5 gm. of soil to 100,000 a gram. This variation in numbers seemed to be related to the absence of bases as indicated by the soil reaction. A soil with a reaction further from neutrality than pH 6.0 did not support as large a number of these organisms as one the reaction of which was nearer neutrality.

The findings led to further studies of the effect on nodulation of supplementing the legume bacteria of the soil with artificial cultures (12) and of the value of such cultures as measured by increased crop yields (15). From these studies it was concluded that certain species of Rhizobium may largely or entirely disappear from soil, that they do not seem to be greatly influenced by the frequency of the host plant in the rotation, and that to acid soils in particular the addition of more legume bacteria has resulted not only in the formation of a larger number of nodules on young plants but also, in certain cases, in a decided increase in crop yields. It was also observed that the numerically increased nodulation on plants of *Pisum sativum* due to supplementary bacteria became less marked as the plants became older until at blooming time there were decidedly more nodules on the plants which grew in soil where the artificial cultures was not used. This may be taken to indicate a seasonal variation in the number of this genus of bacteria.

The foregoing determinations of numbers of Rhizobium and the observations relating to the use of artificial cultures were so striking that it was thought desirable to make a study of the seasonal variation in the number of *Rhizobium trifolii* and of *Rhizobium leguminosarum*, with the view of contributing to our knowledge concerning the subject of supplementary bacteria in relation to the growth of crops.

METHODS

The method of making such a study was essentially the same as that given in a previous publication (13). Samples were taken from .003-acre plats. Quart jars were used to hold a composite sample which consisted of 10 to 12 small samples from the first 3 to 4 inches of soil. To insure uniformity, the composite was well mixed on paper, after which a moisture determination was made. While this was being done the composites were kept in an ice box. In order that the results would be comparable, enough moist sample was taken to give 100 gm. of dry soil. This was then titurated with water in a mortar or dispersed with a mechanical agitator. Dilutions were made from the muddy suspensions. Care was exercised throughout the work to avoid contamination with the legume organism. Portions of the suspensions which were equivalent to a certain quantity of soil, were put on the surface of sterile soil in tumblers where the host plant was to be grown. The tumblers, which held about 200 gm. of sandy soil, were kept tightly covered with paper, which was removed after the plantlets were in need of light. After this period of growth, only 10 or 12 days was required for nodulation to occur sufficiently to determine whether or not the legume organism was present in the quantity of soil used. If contamination occurred after the paper was removed it did not influence the results. Numerous checks were provided and in no case did nodulation occur on the plants in them.

The samples were examined for *Rhizobium leguminosarum* and for *R. trifolii*. To determine the approximate dilutions required for such an examination preliminary tests were made. After suitable dilutions were found they were used in the work herein reported and were modified whenever necessary.

Since it was known that both red clover seed (*Trifolium pratense*) and vetch seed (*Vicia villosa*) carry as contamination (14) the legume organism, the seeds were treated, before being planted, either with a solution of calcium hypochlorite or with concentrated sulfuric acid. Heavy seeding was practiced in order to have adequate plants for inspection. Seeding was usually delayed for 3 to 10 days to permit the establishment of a high legume bacteria content in case the organism was present. After the treated seeds were spread on the surface of the soil in the tumblers they were covered with sterile soil.

The reaction of the soil composite from each plat was determined by the quinhydrone method.

HISTORY OF PLATS

In 1925 and for 9 years previously, plats 751 B, 751 C, 757 B, and 757 C were planted yearly to rye, whereas plats 756 B, 756 C, 756 E, 756 F, 762 B, and 762 C, were planted yearly to oats. In 1926, corn was grown on all of the plats, and in 1927 oats. In 1928, plats 751 B, 756 B, 757 B, and 762 B grew field peas, whereas plats 751 C, 756 C, 757 C, and 762 C grew red clover, and 757 E and F grew cowpeas. Previous to this study the soils of these plats were

known to contain both *Rhizobium trifolii* and *R. leguminosarum*. In the spring of 1928, plats 751 B and 756 B, which grew field peas, had the organism for this crop supplemented with an artificial culture placed on the seed at seedtime. Also plats 751 C and 756 C, which grew red clover in 1928, had their legume

TABLE 1

Seasonal variation in the number of Rhizobium trifolii

Table shows percentage moisture when sample was taken and approximate number of bacteria per gram dry soil.

PLAT	REACTION	TIME OF YEAR WHEN SOILS WERE EXAMINED 1928–29								
			October 11	November 13	December 11	January 22	February 28*	April 11	May 7	June 3
	pH									
751 B	7.50	Per cent	22	22	26	23	39	21	25	17
		Number	1,000	20,000	2,500	20,000	100,000	2,500	10,000	50,000
751 C	7.40	Per cent	12	23	26	23	36	21	25	18
		Number	10,000	2,500	2,500	20,000	20,000	25,000	7,500	25,000
756 B	4.95	Per cent	11	12	23	23	21	18	24	14
		Number	100	500	1,000	100	500	50	10,000	100,000
756 C	4.96	Per cent	10	15	22	20	20	21	24	15
		Number	10,000	750	5,000	10,000	20,000	25,000	500,000	500,000
756 E	4.83	Per cent	10	19	24	23	19	19	24	14
		Number	100	100	100	10	10	10	1 in 1 g.	1 in 5 g.
756 F	4.86	Per cent	10	13	23	21	15	19	24	14
		Number	100	100	100	10	10	10	1 in 5 g.	1 in 2 g.
757 B	7.27	Per cent	14	22	26	25	27	21	25	17
		Number	5,000	750	5,000	1,000	100,000	25,000	75,000	5,000
757 C	7.31	Per cent	15	22	26	24	23	21	25	17
		Number	10,000	5,000	20,000	20,000	100,000	50,000	50,000	100,000
762 B	4.88	Per cent	11	15	23	22	23	20	25	15
		Number	1,000	100	100	10,000	20,000	25,000	25,000	10,000
762 C	4.92	Per cent	22	20	23	23	12	20	25	15
		Number	100,000	100	2,500	7,500	500	50,000	75,000	10,000

* Ground frozen when samples were taken.

bacteria population supplemented in the same way. Since 1926, all crops have been removed from the plats. In October, 1928, all plats were plowed.

In 1922, plats 751 B, 751 C, 756 B, and 756 C received limestone at the rate of 4,000 pounds an acre, and in 1927 they were again limed at the rate of

2,200 pounds an acre. Other treatments, consisting of potassium chloride and acid phosphate which were applied several years before this study was begun, were given to all plats alike.

RESULTS

The first studies which related to the seasonal variations in the total number of any species of Rhizobium in soils were made in 1926. In the following year, additional studies were made. These gave some idea of the number of organisms that may be expected to be present and permitted the development of a technique which was applicable to the work. Beginning in September, 1928, samples were collected regularly about once a month until June 3, 1929. This covered a period when there was no very actively growing plants on the soil. The seasonal variation of *Rhizobium trifolii* is given in table 1.

TABLE 2

Seasonal variation in the number of Rhizobium leguminosarum

Figures indicate approximate number to each gram dry soil

PLAT	TIME OF YEAR WHEN SOILS WERE EXAMINED 1928–29							
	October 11	November 13	December 11	January 22	February 28†	April 11	May 7	June 3
751 B*	50,000	20,000	20,000	10,000	20,000	2,500	25,000	75,000
751 C	10,000	20,000	750	2,500	20,000	2,500	7,500	10,000
756 B	50,000	5,000	10,000	20,000	100,000	5,000	75,000	100,000
756 C	10,000	1,000	500	250	250	100	100	250
756 E	250	100	500	100	20	100	1 in 5 g.	1 in 50 g.
756 F	100	250	500	50	500	10	1 in 5 g.	1 in 2 g.
757 B	5,000	5,000	10,000	1,000	10,000	50,000	75,000	10,000
757 C	10,000	500	1,000	20,000	7,500	50,000	25,000	100,000
762 B	5,000	20,000	500	5,000	20,000	25,000	7,500	10,000
762 C	2,500	100	100	2,500	10,000	50	100	5,000

* For percentage of moisture in and reaction of soils see table 1.

† Ground frozen when samples were taken.

The samples which were collected in February were frozen, and the ground remained frozen until about April 1. It should be noted that there is no relation between the soil moisture and the seasonal variation in the number of legume bacteria. What variation is recorded must be assigned to some other factor than moisture even though it varied considerably. Plats 756 E and 756 F are very outstanding in that they have few *Rhizobium trifolii* as compared with plat 757 C or even 756 C, which is within 12 feet of plat 756 E. These two plats at no time during the 8 tests that were made between October and June showed as many as 100 *Rhizobium trifolii* a gram. By January 22, this species had decreased to less than 10 a gram and by June 3 there was probably less than 1 to 5 gm. of soil. This indicates a gradual decrease from October to June. It should be noted that the reaction of these two plats was about pH 4.8.

Other plats did not show such a drastic decline in *Rhizobium trifolii*. There seems to have been a decline in numbers in November and December, a slight rise in January and February, but a decided decrease in April just after a thaw, with an increase from this date to June 3, at which time the numbers were as high as or higher than they were the previous September.

The plats which show quite consistently large counts of *Rhizobium trifolii* are those which have been limed, namely plats 751 B, 751 C, 757 B, 757 C. They have an alkaline reaction.

The samples of soil which were used for the determination of *Rhizobium trifolii* were also used for a determination of *R. leguminosarum*. The seasonal variation is shown in table 2.

It is noted that the soil from plats 756 E and 756 F did not have over 500 *Rhizobium leguminosarum* to a gram at any time between October 11 and June 3, during which time 8 tests were made. There seems to have been a tendency for the organisms to disappear as spring advanced. In June the soil of plat 756 E did not have more than 1 organism in 50 gm. of soil whereas that from 756 F, which adjoins plat 756 E, had not more than 1 organism in 2 gm. The organisms to each gram of soil of plat 756 C were about 10,000 in October, about 250 in January and February, less than 100 in April and May, and about 250 in June. In the remaining plats there seems to have been a decline in the number of organisms in November and December with a tendency for them to increase in numbers in January and February followed by another decline when the soil thawed in April, with a gradual increase from that date to June 3. Excepting plats 756 C, 756 E, and 756 F, the number of *Rhizobium leguminosarum* in June was as large as or larger than it was in October. If the soil of these plats had been examined later in the spring they probably would have shown similar results.

DISCUSSION

The results of this study of the seasonal variation in the number of Rhizobium in field soil suggest that the legume bacteria find certain soils more adapted to their existence than others and that soils whose reaction is as low as pH 4.8, at certain seasons of the year, may have as few *Rhizobium trifolii* and *R. leguminosarum* respectively as 1 in 5 or 1 in 50 gm. of soil. These findings are similar to those reported by Bryan (2), who suggested that when a soil becomes as acid as pH 5 the organism for red clover is killed.

The maximum count of either species of Rhizobium obtained from each of the 10 plats from which 8 samples were taken between October and June did not occur at the time of any one sampling. Since the growth and longevity of bacteria in soil are dependent upon climatic factors, the physical and chemical make-up of the soil, and the associative action of the edaphon contained therein, such results might have been expected. Bacteria respond to environment which may operate to increase or decrease the population that

may be found at any season. Such seasonal variations as have been recorded in this paper which have to do with *Rhizobium trifolii* and *R. leguminosarum* have also been reported for the heterogeneous bacterial flora of the soil by Lyon, Bizzell, and Conn (8), by Conn (3), by Waksman (11), and by many others.

Since the number of *R. trifolii* and *R. leguminosarum* were determined in the same samples of soil a relative comparison of the numbers of these two species is possible. If all the individual determinations for the organisms for each species are added separately and a comparison is made, it will be noted that there are about 24 organisms of *R. trifolii* to every 11 of *R. leguminosarum*—a ratio of 2.18:1. If such a comparison is made between the organisms in the soil of the four alkaline plats, there are about 89 *R. trifolii* to every 67 of *R. leguminosarum*, or a ratio of 1.33:1; and in the six acid plats about 5 of *R. trifolii* to 1 of *R. leguminosarum*, which is a ratio of 5:1. Apparently *R. trifolii* finds these soils a better habitat than *R. leguminosarum* does. These findings are rather interesting because it is generally believed that the host plants (Vicia and Pisum) of *R. leguminosarum* will do better on an acid soil than will the host plants (Trifolium) of *R. trifolii*. They also suggest an explanation for the observed increase (15) in shelled peas which were taken from these plats in 1928 where the normal bacterial flora in the soil for this plant was supplemented at seedtime with an artificial culture.

The preceding findings are in agreement with data previously published on a study of the legume bacteria population of the soil (13) in which it was pointed out that soils varied in their ability to support *Rhizobium melilotii*, the variation ranging from none in 5 gm. to 100,000 to a gram.

CONCLUSIONS

Soil samples were collected from ten .003-acre plats 8 times between October 11, 1928, and June 3, 1929. The history and reaction of the plats are given. The samples were examined for *Rhizobium trifolii* and *R. leguminosarum*. Definite portions of the soils were used as inoculating material for sterilized soil that was held in glass tumblers. Seeds of *Trigolium pratense* and of *Vicia villosa*, after proper treatment to remove any adhering legume bacteria, were planted. The soils in the tumblers were kept suitable for plantlets by adding the necessary water which was free of the legume bacteria.

The containers holding the inoculated and plated soils were placed in the greenhouse, where favorable conditions were maintained for growth and nodulation. In about 15 days after planting, the plant roots were examined for nodules. The presence or absence of nodules resulting from a series of dilutions established the approximate number of legume bacteria to a gram of soil.

The number of Rhizobium organisms of each species studied showed a wide variation. In the soils of plats 756 E and 756 F the number of *R. trifolii* varied from approximately 100 a gram to as few as 1 in 5 gm. The number varied in the other 8 plats from 50 to 100,000 a gram. The number of *R. legumino-*

sarum varied in the soils of plats 756 E and 756 F from 1 in 50 gm. to 500 a gram, whereas in the other 8 plats there were more bacteria of this species. The variation was in one case from 50 to 5,000 a gram of soil and in another case 2,500 to 75,000.

Except in plats 756 E and 756 F there was a decided drop in the number of both species of Rhizobium in all plats as the winter season advanced. This drop did not always occur in the soils of the different plats at the same date. As the temperature increased in the spring and conditions became favorable for growth and multiplication, the bacteria of both species increased until they were in most cases as numerous in June as they were in October.

REFERENCES

(1) BROWN, P. E., AND HALVERSEN, W. V. 1919 Effect of seasonal conditions and soil treatment on bacteria and molds in soil. *Iowa Agr. Exp. Sta. Res. Bul.* 56: 251–278.

(2) BRYAN, O. C. 1923 Effects of acid soils on nodule-forming bacteria. *Soil Sci.* 15: 37–40.

(3) CONN, H. J. 1914 Bacteria in frozen soil. N. Y. Agr. Exp. Sta. Tech. Bul. 35.

(4) FRANCÉ, R. H. 1913 Das Edaphon. Munich.

(5) GILTNER, W., AND LONGWORTHY, H. V. 1916 Some factors influencing the longevity of soil microörganisms subjected to desiccation with special reference to soil solution. *Jour. Agr. Res.* 5: 927–942.

(6) HARDER, E. C. 1916 The occurrence of bacteria in frozen soil. *Bot. Gaz.* 61: 507–517.

(7) JONES, D. H., AND MURDOCK, F. G. 1919 Quantitative and qualitative bacterial analysis of soil samples taken in fall of 1918. *Soil Sci.* 8: 259–267.

(8) LYON, T. L., BIZZELL, J. A., AND CONN, H. J. 1913 An examination of some more productive and some less productive sections of a field. N. Y. (Cornell) Agr. Exp. Sta. Bul. 338.

(9) VANDERLECK, J. 1917, 1918 Bacteria in frozen soils in Quebec I, II. *Proc. and Trans. Royal Soc. Canada* 3 (ser. 11, sec. IV): 15–37; 3 (ser. 12, sect. IV, V): 1–21.

(10) VASS, A. F. 1919 The influence of low temperature on soil bacteria. C. U. Mem. 27: 1043–1079.

(11) WAKSMAN, S. A. 1916 Bacterial numbers in soils, at different depths, and in different seasons of the year. *Soil Sci.* 1: 363–380.

(12) WILSON, J. K. 1926 Effect on nodulation of supplementing the legume bacteria of the soil with artificial cultures. *Jour. Amer. Soc. Agron.* 18: 280–294.

(13) WILSON, J. K. 1926 Legume bacteria population of the soil. *Jour. Amer. Soc. Agron.* 18: 911–919.

(14) WILSON, J. K. 1929 The presence of Rhizobium on agricultural seed. *Jour. Amer. Soc. Agron.* 21: 810–814.

(15) WILSON, J. K., AND LELAND, E. W. 1929 The value of supplementary bacteria for legumes. *Jour. Amer. Soc. Agron.* 21: 574–586.

INTERRELATION OF NUTRIENTS AND SOIL REACTION ON GROWTH AND INOCULATION OF ALFALFA[1]

M. C. SEWELL AND P. L. GAINEY[2]

Kansas Agricultural Experiment Station

Received for publication March 3, 1930

In crop management there are two important questions pertaining to the production of alfalfa on acid soils: first, what degree of acidity limits the growth of alfalfa provided there is an abundant supply of nutrients; and second, what degree of acidity limits inoculation and hence the nitrogen fixed? The latter question is of particular importance where alfalfa is grown for soil improvement as well as for a hay crop.

In order to throw some light upon these questions, several greenhouse experiments have been conducted and are herein summarized.

The first experiment was planned to show whether alfalfa would grow in an acid medium provided it was well supplied with the necessary soil nutrients. To this end, quartz sand cultures were used with nutrient solutions, uniform as to elements and molecular concentration but varying in degree of acidity. The salts contained in the solutions were $Ca(NO_3)_2$, KH_2PO_4, K_2HPO_4, and $MgSO_4$. Traces of ferric phosphate were used to supply iron.

The nutrient solutions were maintained at three different degrees of acidity, namely at pH values of 4.5, 6.0, and 7.0. The following quantities per liter of the various soils solutions were used in order to obtain these reactions:

pH OF SOLUTION	*M* $Ca(NO_3)_2 \cdot 4H_2O$	*M* KH_2PO_4	*M* K_2HPO_4	*M* $MgSO_4 \cdot 7H_2O$
	cc.	*cc.*	*cc.*	*cc.*
4.5	9.0	4.5	0	4.5
6.0	9.0	3.5	1.0	4.5
7.0	9.0	0	4.5	4.5

The alfalfa was inoculated and planted in 4-gallon jars A glass tube with glass wool to protect the lower end was inserted in each jar before 24 kgm. of sand was put in. Five jars were maintained at each reaction and plants were finally thinned to five to each jar. A wax seal was applied to check evaporation. A cone in the center of the jar permitted additions of fresh solution. Solutions were changed twice each week, being withdrawn by air suction.

[1] Contribution no. 191 from the department of agronomy and contribution no. 125 from the department of bacteriology.

[2] Associate professor of soils and professor of bacteriology, respectively.

The withdrawn solutions were tested colorimetrically for any changes in reaction. The reactions were easily maintained within a few tenths of the indicated pH value.

Plate 1, figure 1, represents the growth of alfalfa with the three conditions of reaction. As is shown, alfalfa grew well in a solution having a pH value as low as 4.5 when well supplied with nutrient elements including, of course, calcium.

The second experiment was designed to show the effect of reaction upon symbiotic nitrogen fixation, as measured by growth of alfalfa, where no nitrogen was supplied in the nutrient solutions. Accordingly other quartz sand cultures were prepared as described in the first experiment. To eliminate nitrogen and to vary the acidity it was necessary to use $CaH_4(PO_4)_2$ and $Ca_2H_2(PO_4)_2$. Potassium was supplied as K_2SO_4, and $MgSO_4$ was used as in the first experiment. The reactions were maintained at pH 4.0, 6.0, and 7.5.

A nutrient solution supplying nitrogen was added until the alfalfa was about 2 weeks old, after which the nutrient solution without nitrogen was substituted.

The effect of reaction when nitrogen is deficient is shown in figure 2, plate 1. After 3 months' growth the plants were washed out of the sand cultures. In the cultures maintained at pH 4.0, the plant roots were stunted and dying, although the top growth was about the same as at pH 6.0 and pH 7.5. The most striking differences evident were in nodulation. At pH 4, there was but one small cluster of nodules in the five plants. At pH 6.0, comparatively few points of inoculation occurred but at these the nodules were present as large grape-like clusters. At pH 7.5, there were a large number of well-distributed small nodules.

These results demonstrate the need of investigation to determine the relationship between reaction with deficient nutrients and symbiotic fixation by the alfalfa plant.

Experiment 3 was a pot culture test with 10 different soils of varying acidities from southeastern Kansas. Alfalfa seed was inoculated and planted in these soils in battery jars. Each soil received the following treatments: (*a*) no treatment; (*b*) lime alone; (*c*) superphosphate alone; (*d*) lime and phosphate. The plants were allowed to grow about 4 months and then the roots were examined. In untreated soils, known to be deficient in calcium and highly acid, no nodules were present on the roots. Where lime alone had been applied, there were a few nodules. Plants grown in soil receiving superphosphate alone showed better nodulation than where lime alone had been applied. With applications of both lime and superphosphate, nodulation as well as total growth was best.

The experiment shows that a supply of available phosphorus is very important in determining the degree of inoculation obtained with the alfalfa plant grown in a normally acid soil.

In the fourth experiment, alfalfa was grown in an acid soil in 42 large cylinders holding about 165 pounds each, to which varying proportions of lime, superphosphate, and potassium sulfate were added.

Plate 2 shows the effect of the lime and phosphate treatments on growth of alfalfa in this soil. In the light of the results portrayed in plate 1, figure 1, the indications are that the differences in growth between cylinders 1, 2, and 3 are due to some physiological effect of lime upon either the plant or the legume bacteria or both, rather than to its effect upon the reaction of the soil. It is true that the reaction was changed from pH 5.0 to pH 6.5 with the lime treatments. However, figure 1 of plate 1 shows that alfalfa will grow well at pH 4.5, provided the plants receive all the needed nutrient elements. Hence it may be inferred that the increased growth of alfalfa in cylinders 2 and 3, compared with cylinder 1, is due to some other effect of the added calcium.

Cylinder 21 received the same amount of lime as cylinder 3 and in addition superphosphate at the rate of 450 pounds an acre. The reaction of the soil in this cylinder was approximately the same as that in cylinder 3. Here again it is very evident that the addition of a nutritive element, phosphorus, rather than a change in reaction caused the additional growth.

Further proof that nutrients more than soil reaction can affect the growth of alfalfa is presented in plate 3. In this photograph all the cylinders received the same lime treatment, 2,000 pounds an acre, and had practically the same reaction. The variable was superphosphate. Cylinder 3 received no phosphate and cylinders 9, 15, and 21, the phosphate increases in the order given. The beneficial effect of phosphorus here may have been due to the nutritive value of the phosphate or, as indicated in experiment 3, to better nodulation.

The limited data presented in this report indicate, that the reaction of the soil, if within a range of pH 4.5 to 7.0, is a minor factor in affecting growth of alfalfa, all the needed nutrients being supplied; that the reaction may be a very important factor in symbiotic fixation, under conditions of limited nutrients; and that variation in nutrients with constant reactions causes great variation in the growth of alfalfa, which may be attributed in part to the effect of the nutrient on nodulation and fixation of nitrogen.

PLATE 1

FIG. 1. Growth of alfalfa in quartz sand cultures supplied with solutions varying in degree of acidity.

a. Solutions having a pH value of 4.5

b. Solutions having a pH value of 6.0

c. Solutions having a pH value of 7.0

FIG. 2. Effect of degree of acidity on inoculation.

a. Solution having a pH value of 4.0

b. Solution having a pH value of 6.0

c. Solution having a pH value of 7.5

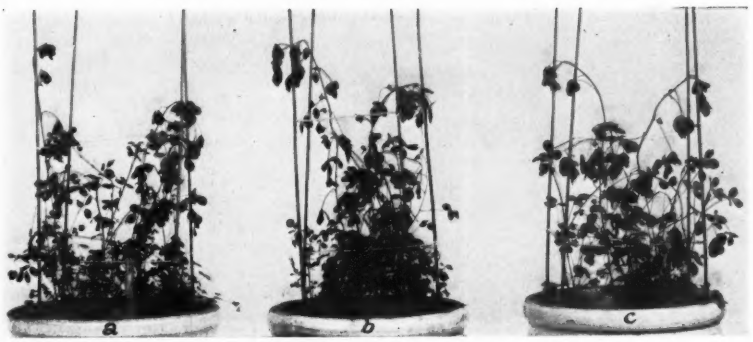


FIG. 1

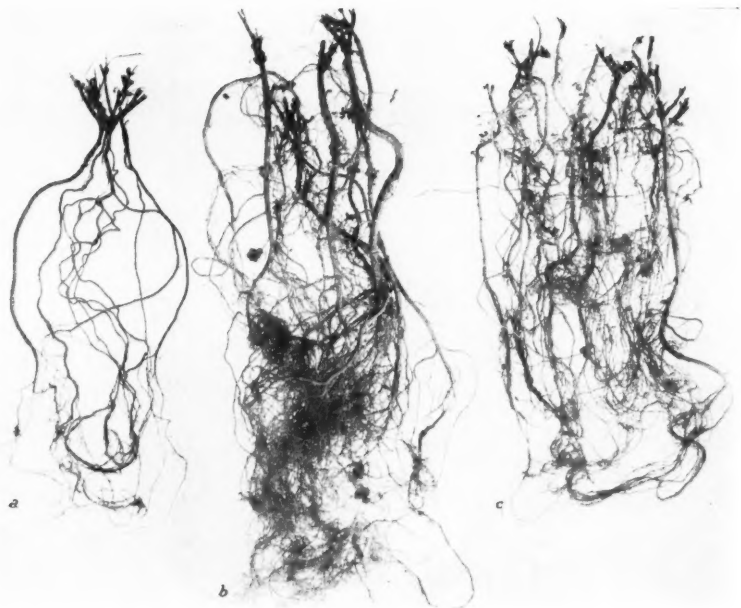


FIG. 2

PLATE 2

EFFECT OF LIME AND PHOSPHATE TREATMENTS ON GROWTH OF ALFALFA IN AN ACID SOIL

Cylinder 1. No treatment
Cylinder 2. Lime, 1,000 pounds an acre.
Cylinder 3. Lime, 2,000 pounds an acre.
Cylinder 21. Lime, 2,000 pounds an acre plus superphosphate 450 pounds an acre.

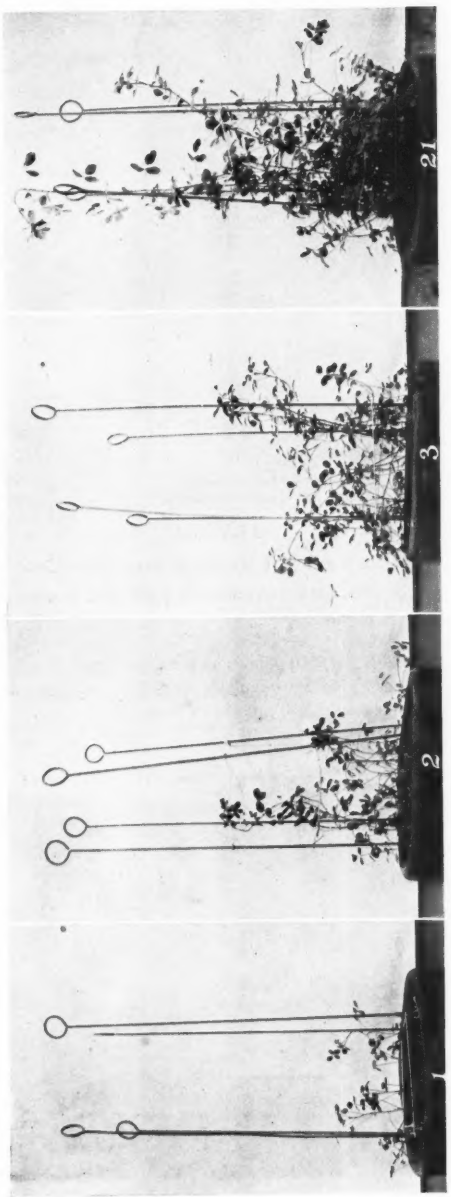
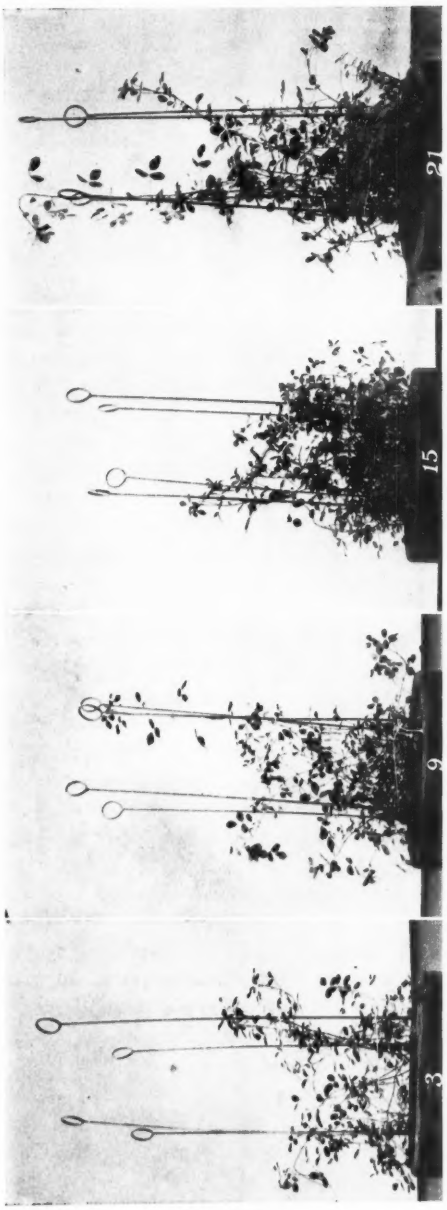

21
3
2
1

PLATE 3

How Nutrients More Than Soil Reaction Affect the Growth of Alfalfa

All cylinders have the same liming treatments—2,000 pounds an acre. In addition, the following treatments were given:

Cylinder 3. No phosphate.
Cylinder 9. Superphosphate, 150 pounds an acre.
Cylinder 15. Superphosphate, 300 pounds an acre.
Cylinder 21. Superphosphate, 450 pounds an acre.


21
15
9
3

DETERMINATION OF CARBONATES IN SOIL

C. J. SCHOLLENBERGER

Ohio Agricultural Experiment Station

Received for publication March 4, 1930

The determination of carbonate native to calcareous soil or residual from liming material applied to acid soil is often required in soil studies. As evidence of the long-continued interest in this determination may be cited the numerous methods described in the literature of soil chemistry. At present there is no procedure which is accepted as standard by American workers. Some of those in use are admitted to be inaccurate, others are too time-consuming or require glass parts that must be specially made or are very complicated. There seems to be a real need for an accurate method that can be carried out in a short time, requires a minimum of apparatus of a kind likely to be found in any well-stocked laboratory or that can be made without difficulty by one with a little skill at glassworking, and is so simple that no elaborate precautions are necessary for high accuracy.

In principle, the estimation of carbonate in soil presents no difficulties; it is only necessary to treat soil with dilute acid to decompose the carbonate minerals and determine the carbon dioxide evolved by any suitable method. However, because of the presence in all soils of organic matter, which is more or less oxidized or decomposed with separation of carbon dioxide in the presence of any considerable concentration of acid, some standard methods for the determination of carbonates are not adapted for use with soils. This action is greatly accelerated by increase in temperature, hence any method which includes boiling the sample with acid at atmospheric pressure for expulsion of carbon dioxide will be grossly inaccurate when applied to soils. This fact is generally recognized, so that aeration with a stream of purified air, often with vigorous agitation as well, to remove evolved carbon dioxide from the soil and dilute acid mixture, is a feature of most methods proposed in recent years.

Aeration is especially adapted to collection of carbon dioxide in a weighed absorption apparatus for a gravimetric determination. Such a procedure is in almost universal use for combustion work but is less satisfactory in the determination of carbonates in soil. To remove carbon dioxide from the dilute acid and soil mixture requires considerable time and a large volume of air aspirated through the solution, which carries considerable moisture into the purifying train. The desiccants used in this require frequent renewal, and there is constant uncertainty as to whether the absorption apparatus may be gaining or losing moisture. At best, it is difficult to weigh the heavy absorp-

tion apparatus with accuracy, so that the theoretical advantages of the gravimetric procedure are seldom realized in practice. Clark and Collins (3) have recently described such a method, and state that from 2 to 7 hours may be required for the absorption apparatus to come to constant weight. In the description of the present tentative method of the Association of Official Agricultural Chemists for carbonates in soil (2), it is stated that 30 minutes aspiration is sufficient, except with soils containing dolomite or limestone resistant to solution. The latter is a volumetric method. In both procedures the carbonate in the sample is decomposed by hydrochloric acid at 1:10 dilution at room temperature and carbon dioxide removed by aspiration with agitation.

With methods employing continuous aspiration there is no means of determining when the last traces of carbonate mineral are decomposed and all carbon dioxide removed. Dolomite or other forms of limestone soluble with difficulty in 1:10 hydrochloric acid at room temperature are of frequent occurrence in the soil in many regions, and are also used as liming materials. It would be very desirable to have some visible indication of completion or decomposition and absorption, as this would result in saving time with samples containing no carbonate or only easily soluble carbonates and would avoid inaccuracy with samples containing resistant limestone. The procedure about to be described has this advantage, as the behavior of the acid and soil boiling in vacuo is characteristic as soon as carbonates are practically completely dissolved, and the great expansion of the bubbles of carbon dioxide arising from even the smallest particle of undecomposed limestone renders them easily visible. The absorption of carbon dioxide by barium hydroxide solution results in a visible film of precipitate on the surface of the solution, a fairly delicate test for completion of absorption. By allowing a short time beyond these points, there can be no doubt that all carbon dioxide which can be obtained has been absorbed. Decomposition and absorption are accomplished in a closed system from which carbon dioxide cannot escape, nor enter from the outside if the details of the procedure are followed. A very accurate titration procedure for the determination of carbon dioxide has been developed. In addition to the foregoing, some hitherto unsuspected sources of error in the determination of soil carbonates are pointed out, and means for overcoming them described.

REVIEW OF PREVIOUS WORK

Probably the most rapid and effective method for the removal of carbon dioxide from sample and dilute acid that can be devised is by boiling in vacuo. As it is possible to accomplish this at a temperature only slightly above that of the laboratory, it is especially adapted to work with soils. A method based on this principle was proposed by Marr (6), and in modified form has long been in use in this laboratory. Gaither (4) substituted a bead tower for the Reiset apparatus employed by Marr, but retained the double titration procedure for the determination of carbon dioxide absorbed by sodium hydroxide solution. Later, carbon dioxide was absorbed in a measured excess of standard barium hydroxide solution in a Meyer absorption apparatus and determined either by filtration and washing, with subsequent

solution in standard acid and titration, or by titrating the excess of barium hydroxide in the presence of the precipitate of carbonate with standard hydrochloric acid and phenolphthalein as indicator (8). The latter procedure has been studied by Truog (10) and shown to be preferable to absorption in sodium hydroxide and determination by double titration. Recently, thymolphthalein has been substituted in the titration of excess barium hydroxide in the presence of precipitated carbonate (9). Long experience with this procedure of titrating excess barium hydroxide has convinced the author that it is amply accurate for all ordinary requirements with only the simplest precautions, and has important advantages over other methods for the determination of carbon dioxide obtained from soil by treatment with dilute acid.

As originally described, Marr's apparatus and the modifications developed later were supposed to require almost continuous operation of the air pump, in order to maintain the vacuum by drawing evolved carbon dioxide through the absorbent solution; none of the designs offered opportunity for absorption in any other way. It was found that there was considerable danger of carbon dioxide being drawn through the absorbent solution and lost, on account of the tendency of this solution to boil because it is under lower pressure than the solution in the evolution flask. This necessitated considerable care and attention. All the forms of apparatus are also of complicated design and require special parts, with numerous joints at which leaks are troublesome. Probably for these reasons, and because it was thought that the use of any heat, however slight, is undesirable in the determination of carbonate in soil, a procedure including the essential feature of decomposition by very dilute acid, boiling and absorption in vacuo, which had been considered as a tentative method by the Association of Official Agricultural Chemists (1), was not retained.

Hutchinson and MacLennan (5) proposed a method based upon that of Marr, requiring only an extremely simple apparatus, consisting of two flasks connected by a wide tube with a bulb to stop foam or spray and stopcock funnels for the introduction of reagents. In their procedure, the sample is placed in the smaller flask and the apparatus exhausted. The absorbent, sodium hydroxide solution, is allowed to enter the absorption flask, and 1:50 hydrochloric acid is allowed to enter the flask containing the sample, in order to decompose the carbonates. No heat is employed, but the reduction in pressure, aided by vigorous agitation, is relied upon to remove all carbon dioxide from the one flask and ensure its absorption in the other. The originators of the method claim that decomposition and absorption can be accomplished in from 20 to 60 minutes.

The author has found that in a similar apparatus and procedure, it is possible to expel all carbon dioxide from the evolution flask and ensure its absorption in barium hydroxide solution in the other flask, within 8 minutes *after all carbonates have been dissolved*, provided the acid and soil mixture is warmed sufficiently to boil at the pressure within the apparatus. This pressure is easily reduced and maintained at a point corresponding to the vapor pressure of water at room temperature, so that the actual temperature in the boiling flask need be but very little higher than that of the room, and may be even lower if a condenser with very cold water is used to connect the flasks. The addition of a condenser to the apparatus described by Hutchinson and MacLennan, therefore, enables one to carry out the procedure of Marr under more favorable operating conditions than were possible with the original apparatus or any modification of it so far proposed. By some other slight changes, the space occupied by the apparatus can be reduced and all the operations facilitated. The most important results of the substitution of absorption of carbon dioxide by exposure to a surface of absorbent solution for absorption by bubbling through the solution are, first, reduction in boiling temperature, and second, insurance of complete absorption. The first result follows from the greater vacuum obtainable in the boiling flask, because the vacuum pump does not work against the hydrostatic pressure of a column of absorbent solution. In the original procedure of Marr, the temperature at which carbonates are decomposed is approximately 50°C. In the Meyer apparatus, the height of liquid is less, and the temperature in the flask with this modification is about 40°C. By using a plain flask as the container for the absorptive solution, the temperature in the boiling flask may be kept without difficulty within the range of 25 to 30°C.

EXPERIMENTAL

Lowering the temperature of expulsion of carbon dioxide is advantageous with respect to lessening attack upon organic matter but is attended by reduction in speed of decomposition of dolomitic limestone. This speed can be increased by using stronger acid, but possibly with the penalty of a corresponding increment of carbon dioxide from organic matter. It should be possible to strike a balance between these factors, and find a procedure which would decompose carbonates quickly with the minimum production of carbon dioxide from sources other than carbonates. With this idea in mind, experiments were undertaken to obtain some definite data on the problem.

The source of the carbon dioxide obtained by the action of acid upon soil which would not be expected to contain any carbonate minerals has been variously explained, but usually vaguely ascribed to decomposition of organic matter. It has been observed that the action of comparatively strong hydrochloric acid is greater than that of the same reagent when highly diluted, or of less dissociated acids such as phosphoric or acetic. No suggestion as to the actual reaction involved has ever been offered, to the author's knowledge. It is his belief that the reaction is largely an oxidation of carbonaceous materials by highly oxidized minerals native to the soil, especially the higher oxides of manganese. Manganese dioxide has been shown to be a common constituent of soils (7). Those soils which contain most, as indicated by ability to decompose 15 per cent hydrogen peroxide, with abundant organic matter also, evolve most carbon dioxide from sources other than carbonates upon treatment with stronger hydrochloric acid or at higher temperature. Addition of chemically pure powdered manganese dioxide to soil and acid results in an increase in carbon dioxide production. In the presence of an effective reducing agent for manganese dioxide, e.g., ferrous chloride, the non-carbonate carbon dioxide obtained from soil upon treatment with dilute hydrochloric acid is greatly diminished.

Hydrochloric acid is the most effective solvent for the carbonate minerals which may occur in soil, but it also reacts with manganese dioxide to form free chlorine, a very active oxidant. This is doubtless the explanation of the fact that hydrochloric acid is apparently more active in decomposing organic matter with production of carbon dioxide than is phosphoric acid, for example. As has just been mentioned, however, addition of ferrous chloride reduces oxidation of organic matter to an almost negligible amount, so that hydrochloric acid plus ferrous chloride is the most suitable reagent for the decomposition of carbonates. From a consideration of data presented in table 1, it is seen that even in the presence of ferrous chloride as antitoxidant, increase in either time, temperature, or strength of acid causes slightly higher results, so that it is evidently necessary to operate as near room temperature and with the most dilute acid possible, consistent with complete solution of carbonate in a reasonable period of time.

Experiments were conducted with samples of calcite and dolomite, considered

to be representative of extremes in ease of solution of carbonate minerals likely to be present in soil. Both were ground and sieved to pass 100 mesh and

TABLE 1

Calcium carbonate equivalent of CO_2 obtained from soils by modifications of Marr's method

SAMPLE	(1) 1:50 HCl MEYER APPARATUS	(2) 1:50 HCl FLASK	(3) 1:50 HCl + $FeCl_3$ FLASK	(4) 1:10 HCl FLASK	(5) 1:10 HCl + $FeCl_3$ FLASK	RECOVERY OF CO_2 FROM 0.2500 DOLOMITE ADDED TO 20 GM. SOIL GM. $CaCO_3$ (THEORY 0.2700)
	m.e./100 gm.	*m.e./100 gm.*	*m.e./100 gm.*	*m.e./100 gm.*	*m.e./100 gm.*	
Wooster silt loam, virgin: 0–1½ inches, pH 5.1, 173 ml. O_2*	1.61	0.43(23°)	0.18(27°)	2.91(28°)	0.25(28°)	0.2705(30°) 0.2712(28°)
1½–5 inches, pH 4.8, 94 ml. O_2*	0.83	0.15(31°)	....(27°)	0.42(27°) 0.40(27°)	....(27°)	
Cultivated, 0–7 inches, pH 4.5,11 ml. O_2*	0.05	....(28°)		....(28°) 1.13(76°)		
Same plus 2.5 per cent MnO_2		....(28°)	....(28°)	0.15(28°) 0.08(28°)	....(27°)	
Canfield silt loam, virgin, 1½–3 inches, pH 5.6, 98 ml. O_2*	1.77	0.15(28°)	0.05(28°)	0.93(28°)	0.13(29°)	0.2705(30°)
Ellsworth silt loam, virgin, 0–2 inches, pH 5.4, 50 ml. O_2*	1.15	0.28(28°)	0.10(27°)	0.73(28°)	0.18(28°)	0.2705(33°)
Peat, pH 5.2, 38 ml. O_2*		0.70(27°) 0.75(31°)	0.60(27°)	1.25(28°) 1.10(34°)	1.00(28°) 6.50(100°)	0.2690(30°)
Peat plus 2.5 per cent MnO_2		0.70(26°) 0.70(24°)	0.60(26°)	1.85(26°) 3.65(28°) 23.5 (100°)	1.00(28°)	
Hawaiian manganiferous soil, pH 5.5†		1.20(36°)	0.05(28°)	1.60(29°)	0.30(28°)	
Toledo silty clay, 0–3½ inches, pH 7.1, 3 ml. O_2*		7.43(31°)	7.25(28°)	7.28(31°)	7.13(28°)	0.2697(30°)
Clermont silt loam, 108–120 inches, pH 8.2, 8 ml. O_2*			32.18 per cent		32.20 per cent	

* Reaction determined on 1:1 suspension by use of quinhydrone electrode. Oxygen evolved on treating 1 gm. soil with 20 ml. 15 per cent H_2O_2 for 10 minutes, similar to test proposed by Robinson (7) for presence of MnO_2 in soil.

† Reaction determined as above by use of antimony electrode. Rate of oxygen evolution too great for measurement.

remain on 150 mesh, washed to free from dust, and dried. This particle size was chosen because it is the maximum likely to be present in a soil sample properly prepared for analysis, although it is scarcely practical to grind any

considerable number of samples to uniform fineness greater than this. It was found that 0.25-gm. charges of the calcite were very readily dissolved by 100 ml. of hydrochloric acid at 1:50 dilution (0.23*N*) at 25 to 30°C., solution being complete in about 2 minutes. The rate of solution was slightly retarded by addition of 5 ml. of ferrous chloride solution, about 3 minutes being required. Mixed with 20 gm. of carbonate-free soil, the rate of solution was still less, 5 or 6 minutes being required when solution was aided by boiling in vacuo, within the temperature limits given. Dolomite was very slowly attacked by the reagent at the strength specified, requiring over an hour for solution in the plain acid and nearly 3 hours in the presence of soil and ferrous chloride. By increasing the strength of acid to 1:10 dilution (1.17*N*), it was found possible to insure complete solution of the dolomite in the presence of soil and ferrous chloride in 30 to 35 minutes boiling at 25 to 30°C.

Although data in table 1 appear to indicate that the use of the stronger acid may lead to results slightly too high, it is not certain that the increase in time of action which would have been necessary had the soils contained dolomite, would not have led to plus errors as great even with the more dilute reagent. In the case of the single soil investigated which is known to contain native dolomite, the Toledo silty clay, the results obtained by the use of 1:50 hydrochloric acid, requiring 1½ hours for complete decomposition, are slightly higher than those from the use of 1:10 acid, which decomposed all carbonates in 45 minutes. This soil is high in organic matter, but differs from most of the others studied in that the manganese dioxide content is low, so that the addition of ferrous chloride had little effect upon the results. In this instance, at least, the use of the stronger acid not only resulted in a saving in time, but it apparently decreased attack upon organic matter as well. In other cases where the stronger acid was used with carbonate-free soil, it was observed that practically all the carbon dioxide was obtained during the first few minutes, hence it seems doubtful whether the aforementioned circumstance would be of general occurrence.

In explanation of table 1, it may be said that with three or four exceptions, the soils were selected because they are very acid, but indicate an appreciable carbonate content by the method that has been in use. The results by this method, given in column 1, were obtained by 15 minutes boiling in vacuo with 1:50 hydrochloric acid and absorption of carbon dioxide in barium hydroxide solution, followed by titration in the same way as the others. The temperature of the acid and soil mixture during the boiling was not determined, but is thought to have been about 40°C. Data in columns 2 to 5 were obtained by use of the apparatus and procedure shortly to be described. The temperature at the end of the boiling period was determined in each case. Variations are due to differences in temperature of the room or condenser water, size of flame, and slight leaks of air into the apparatus. In a few instances the apparatus was intentionally only partly exhausted, in order to observe the effect of high boiling temperatures. Except in the case of the Toledo silty clay, all deter-

minations with 1:50 hydrochloric acid were made with a 15-minute boiling period, and all determinations in which 1:10 acid was used, with a 45-minute boiling period. In the last column, recoveries of carbon dioxide from 0.25-gm. charges of dolomite added to 20-gm. samples of soil and decomposed by a 45-minute boiling with 1:10 acid and ferrous chloride in vacuo, are tabulated. In each case, the carbon dioxide obtained from a similar experiment without addition of dolomite (column 5) has been deducted as a blank, and the remainder tabulated as the recovery from the added dolomite. Several careful duplicate determinations of carbon dioxide obtained from dolomite without soil indicated that the calcium carbonate equivalent of each 0.25-gm. charge was 0.2700 gm. The average of the determinations in the presence of soil is 0.27023 $\pm$0.00023 gm. calcium carbonate.

The 1½-inch surface layer of the virgin Wooster silt loam contains a large amount of partly decayed organic debris and is also high in active manganese dioxide, as indicated by its ability to decompose 15 per cent hydrogen peroxide. Every modification of procedure obtained some carbon dioxide from this sample. When the acid was added to the soil already wetted with water in the evacuated apparatus, there was noted a flash of foam as shown by soils undoubtedly containing a trace of finely divided carbonate, hence it is possible that this soil really contains a trace of carbonate. It is appreciably less acid than the layer immediately below. Use of strong acid on this sample leads to large error, but this is practically eliminated if ferrous chloride is added with the acid. The figures for recovery of carbon dioxide from added dolomite are both high. The 1½ to 5-inch layer of the same profile is indicated to contain no carbonate when ferrous chloride is added with the acid. The manganese dioxide content of this sample is high. The cultivated sample of Wooster silt loam is deficient in organic matter and low in active manganese dioxide, hence it indicates no apparent carbonate content except by treatment with the stronger acid at a higher temperature. Addition of manganese dioxide to this soil causes slightly high results, but the use of ferrous chloride prevents the oxidizing action. The 1½ to 3-inch horizon of the Canfield silt loam is similar to the corresponding sample of the virgin Wooster silt loam, whereas the surface sample of the virgin Ellsworth silt loam contains less organic matter than the Wooster type, and also less active manganese dioxide, but apparently contains a trace of carbonate.

The three virgin samples indicating a slight carbonate content all show the same increase in carbonate because of substitution of 1:10 hydrochloric acid for the 1:50 acid, ferrous chloride being used in each case. This increase amounts to 0.07 or 0.08 m.e. calcium carbonate to 100 gm. soil and undoubtedly represents the effect of the stronger acid and increased time of action upon the soil organic matter. The difference is quite insignificant, but this is not the case when the corresponding figures from experiments without addition of ferrous chloride are compared. Without the antioxidant, increases in time of action or strength of acid have caused considerable increases in apparent car-

bonate content, and the errors so introduced could not be considered negligible. These differences correspond to differences in the ability of the three samples to decompose hydrogen peroxide, pointing to some factor common to both phenomena.

The peat soil is rather on the border of being a muck and contains considerable mineral matter washed from higher ground. In spite of its acid reaction it appears to contain carbonate. The organic matter is apparently little oxidized by manganese dioxide in the presence of the most dilute hydrochloric acid, but is considerably affected by the stronger acid, especially when heated. Increase in strength of acid has a considerable effect *per se*, apparently quite apart from oxidizing action.

The manganiferous soil[1] is stated to be representative of the chocolate brown, highly manganiferous soils of the Hawaiian Islands. It is high in manganese dioxide and decomposes hydrogen peroxide very vigorously, but appears to be deficient in organic matter. This soil is especially interesting as an example of a case in which two independent lines of evidence, usually confirmatory, lead to a false conclusion. By the ordinary method, this soil is indicated to contain considerable carbonate. It is indicated to be alkaline by the quinhydrone electrode, pH 7.3 at the first reading which can be taken, but with potential drifting very rapidly. If reliance were placed upon the two tests, it would be concluded that this soil is alkaline. Nevertheless, determinations of reaction by the hydrogen and antimony electrodes, and color tests with indicators indicate that the soil is quite acid, between pH 5.5 and 5.7, whereas addition of ferrous chloride to the acid used in the carbonate determination results in only a trace of carbon dioxide being obtained.

The limestone in the Clermont silt loam parent material dissolves readily in 1:50 hydrochloric acid, an indication that it is not of dolomitic character. The results indicate that on this sample, the use of stronger acid is not necessary.

Apparatus

The apparatus with accessories is shown in figure 1. The 200-ml. round-bottomed, short, ring necked Pyrex flask *A* is that in which the sample is treated with acid. The similar 1-liter flask *B* is that in which the CO_2 is absorbed and subsequently titrated. The two flasks are connected by the upright water-jacketed condenser tube *C*, at the upper end of which is a large semicircular bend attached as a side arm as indicated by the drawing, which is made to scale. The straight part of the condenser tube, continued as the other limb of the T for a short distance above, is closed by a rubber stopper with inserted glass tube, to which is hung by a wire the gauge *G*, also shown in detail. The inner tube of this gauge was made from a piece of graduated pipette, inside diameter about 3 mm. The graduations on the tube enable one to read the

[1] Supplied by W. T. McGeorge, formerly of the Hawaiian Sugar Planter's Experiment Station.

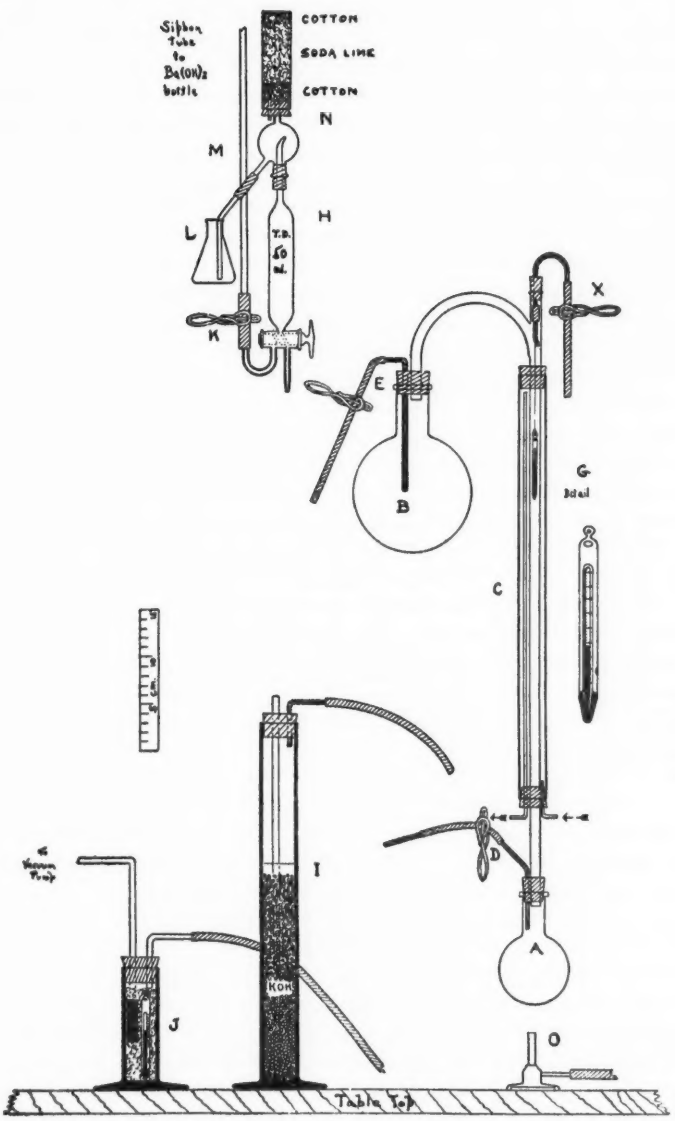


FIG. 1. APPARATUS FOR THE DETERMINATION OF CARBONATES IN SOIL

mercury level with considerable accuracy. Other details of construction will be plain from the drawing. The purpose of the gauge is not so much to indicate the absolute pressure within the apparatus, as to indicate when the apparatus has been exhausted to the practical capacity of the pump, and to give warning of leaks which might otherwise not be suspected. It is not necessary, therefore, to use very great care in filling the gauge with mercury to get every trace of air and moisture from the inner tube. It will suffice to put the mercury in the outer tube, and then put the gauge in a heavy test tube, which is connected to the pump and thoroughly exhausted. By tilting, the mercury is allowed to run away from the tip of the graduated tube, so that there is no hindrance to escape of gas. Under the conditions of practical use, the gauge will never be required to register a vacuum quite as great as this, hence the mercury will always stand at a level where it can be seen and the exact position with reference to the graduations on the tube noted.

The inner tube of the condenser is made from a piece of soft glass tubing, 10 mm. inside diameter and 1 mm. wall thickness. The side arm is of the same tubing. The condenser jacket is a piece of heavy-walled tubing about 25 mm. inside diameter and 45 cm. long. In making the glass parts these dimensions and others which may be taken from the drawing should be followed, as the satisfactory performance of the apparatus may depend upon some of them. The jacket is attached to the condenser tube by means of no. 5 rubber stoppers; both are bored with one large hole slightly off center for the condenser tube, and the lower stopper is bored with two additional holes for glass tubes by which cooling water enters and leaves the condenser.

Especial care should be taken in selecting and boring the two stoppers which connect the flasks to the condenser tube. These stoppers should be of the best quality, red, antimony-cured rubber, selected for soundness and elasticity, and large enough to be forced down into the mouths of the flasks to make a tight joint without risk of being drawn entirely in by air pressure. Each is carefully bored with one large hole 10 mm. in diameter for the condenser tube and one small hole 4 mm. in diameter for the heavy capillary tube of approximately 1 mm. bore for introduction of reagents. These tubes should be long enough to extend down into the flasks as shown. Suitable lengths of 3-mm. heavy-walled tubing are attached to them; this should be stiff enough not to collapse, but not so hard that it cannot be securely closed by the clamps. The most satisfactory clamp is the Day pinchcock pattern.

The apparatus is supported by an upright rod, to which it is secured by one large condenser clamp at about the middle of *C*. A few turns of adhesive tape about the condenser jacket tube and thick rubber tubing on the jaws of the clamp protect the glass from breakage. The adhesive tape enables the clamp to hold the glass tube without slipping and with less pressure than would otherwise be necessary. If many determinations are to be made, two or three assemblies can be fastened to one rod and all shaken at the same time. Because of the brief time required for a determination with most samples, it will

be found that one man's time is fully occupied by this number. An extra set of flasks for each condenser should be at hand.

Accessory apparatus shown in the figure includes the automatic pipette *H* for delivery of a measured volume of $Ba(OH)_2$ solution siphoned through *M* from a stock bottle on a shelf above. The overflow from the pipette is caught in the flask *L*. Guard-tube *N*, filled with coarse soda lime, permits only purified air to enter *H* when emptied, hence the pipette rarely requires cleaning. When this is necessary, it can be accomplished without taking down the apparatus entirely. The top part is removed and the body of the pipette cleaned by drawing through dilute HCl, water, concentrated H_2SO_4 with CrO_3, and is finally very thoroughly washed with water to remove every trace of sulfate before the barium solution is again admitted. Pinchcock *K* is insurance against loss of the entire stock of solution in case the plug should become loose in its shell. *L* and *H* are fastened by clamps to a rod attached to the shelf on which the stock bottle stands, directly above *E*. If only a few determinations are to be made, it will not be worth while to set up an automatic pipette. Excellent results can be obtained by the use of an ordinary transfer pipette, into which the clear $Ba(OH)_2$ solution is drawn considerably above the mark. The tip is wiped, and the level of the solution adjusted, whereupon the pipette is tilted and the solution caused to retreat from the tip, which may then be inserted into the end of *E* and the solution accurately delivered into *B*.

The bead tower *I* is half filled with glass beads with a strong solution of KOH (30 gm. in 100 ml. water) to cover. Air allowed to enter the apparatus at the end of the procedure, before the contents of *B* are titrated, is drawn through this tower for purification.

Trap *J* is intended especially to protect the vacuum pump from liquid which might be drawn into it, and from dust which may rise from dry soil on sudden application of vacuum. The glass tube connected to the pump ends in a cylinder of wire gauze, closely wrapped with cotton. A gauge is enclosed within the trap.

Any type of vacuum pump which will exhaust the apparatus with sufficient speed is suitable. A "Cenco Hyvac" pump has been in use in this work for two years past, and has given very little trouble.

Reagents

Barium hydroxide solution. Prepare a solution by dissolving the chemically pure crystals at the rate of 65 gm. to a liter of boiled and still hot water. Let stand over night for carbonate to settle. This solution is about 0.4 *N* and is practically saturated at room temperature. Siphon the clear solution into the storage bottle, which is already partly filled with the boiled and cooled water required for dilution to the proper strength. This should be such that one filling of the pipette used to deliver the solution will require slightly less than a burette full of the acid used in the titration. The author makes the solution slightly weaker than 0.2 *N*, delivers it from a 50-ml. pipette and titrates with 0.1 *N* HCl from a 100-ml. chamber burette (M. C. A. No. 3).

Tenth-normal hydrochloric acid. A solution standardized by any of the well-known methods may be expected to have very nearly the theoretical value in carbonate, that is, 1 ml. = 0.005 gm. $CaCO_3$. For the highest accuracy, however, it is best to standardize the acid against a sample of pure $CaCO_3$ carried through the procedure. The water used in the preparation of the standard HCl should have been freed from CO_2 and the solution protected from absorption of any from the air.

Carbon dioxide-free water. Boil distilled water in a large flask or metal can for half an hour, and protect it from the air while cooling and during storage. Dissolved air is objectionable in the water and solutions used in the apparatus, as it reduces the vacuum to some extent. Therefore, CO_2 should be expelled from the water by boiling rather than by aeration. Reabsorption of both CO_2 and air by the water and solutions can be prevented by a layer of pure medicinal paraffin oil about 1 cm. thick, covering the surface. This also prevents the evaporation of solutions in partly filled stock bottles and condensation of water on the exposed walls, and so helps to keep the solution uniform in strength.

Dilute hydrochloric acid. Dilute 1 volume of the C.P. concentrated hydrochloric acid to 10 volumes with CO_2-free water. This is the 1:10 HCl previously referred to, and is about 1.2 *N*. The 1:50 acid is made by diluting this solution with 4 volumes of water.

Ferrous chloride solution. Put about half a pound of small wire nails in a 500-ml. flask and cover them with hydrochloric acid diluted with an equal volume of water. Add a drop of "antifoam" to prevent foaming over, and set in the hood until action practically ceases. Pour off into a second flask and boil to expel H_2S. Filter and preserve under oil. One ml. should contain about 0.35 gm. $FeCl_2$ and reduce 0.2 gm. MnO_2.

"Antifoam." A mixture of equal volumes of light mineral oil and capryl alcohol. A drop or two added from a small oiler to mixtures of soil and dilute acid which cause trouble from tenacious foam rising in the condenser, will cause the foam to break quickly.

Indicator. About 0.5 ml. of a 0.5 per cent solution of either phenolphthalein or thymolphthalein in neutral 95 per cent alcohol may be used, but the two cannot be employed indiscriminately. The latter shows a sharper endpoint in the presence of much carbonate, but has the serious defect of a slow color change, hence the titration must be slowly and carefully performed. It also forms a colloidal precipitate in neutral solutions containing little carbonate, and the "tyndall blue" is likely to be mistaken for the alkaline color of the indicator. If the blue color is very faint and is not diminished by an additional drop of acid under these conditions, in all probability the proper endpoint has been reached. A certain check is to add phenolphthalein, which should show a strong pink color requiring 0.15 ml. 0.1 *N* HCl to discharge if the solution is about 200 ml. volume and was exactly neutral to thymolphthalein. If $BaCO_3$ from 0.25 gm. $CaCO_3$ is present, the solution will require the addition of about

0.4 ml. 0.1 *N* HCl to discharge the phenolphthalein color, and this may return on standing, because of loss of CO_2. Therefore, the normality of the acid will depend to a slight extent upon which indicator is used. The difference is to be attributed to solubility and hydrolysis of $BaCO_3$, which affect thymolphthalein less than phenolphthalein, on account of the higher pH value at which the former is decolorized.

Procedure

Weight out a suitable amount of the sample, which may be 20 gm. or more if low in carbonate, but should not contain much more than the equivalent of 0.25 gm. $CaCO_3$. Transfer to flask *A* and connect securely. Connect empty flask *B* and attach clamp *E* near the free end of the rubber tube, and also have clamp *X* attached. Connect flask *A* to the vacuum pump through trap *J*, remove clamp *D* and exhaust the apparatus until gauge *G* indicates about 2 cm. Hg pressure within the apparatus, or the greatest vacuum obtainable with water in the apparatus. A satisfactory pump should do this within two minutes. Near the end of the period of exhaustion, connect rubber tube *E* to the tip of the pipette *H*, the body of which is filled with $Ba(OH)_2$, but with tip and bore of plug empty. Release clamp *E* momentarily, then open the stopcock on *H*. By cautious release of clamp *E*, permit the solution to flow into *B* at about the rate at which such a pipette should be emptied for reproducible delivery, until the last drop of solution has been drawn from the tip of *H*. Close the clamp, then the stopcock, then release the clamp for a moment, in order that the expansion of air in the tip of *H* may drive the solution in the rubber tube *E* into flask *B*. Pinch the rubber tube near its end and remove it from *H*, and without releasing the pressure immerse the end in a 50-ml. beaker filled with CO_2-free water. By releasing clamp *E*, the beaker is emptied rapidly and all $Ba(OH)_2$ washed from the rubber tube *E* into flask *B*. Needless to say, the flow is stopped while the tube is still filled with water. By this time, the apparatus should be so completely exhausted that the liquid just added is boiling or at least evaporating so rapidly that water is beginning to condense near the top of the condenser *C*, through which a plentiful supply of cold water should be circulating. Attach clamp *D* near the short capillary shown at the end of the rubber tube, stop the pump, and disconnect. Allow about 25 ml. carbon dioxide-free water to enter the apparatus at *X*, adding most of this at once, shaking *A* until the sample is wetted, then adding the remainder of the water to wash dust down from the condenser. Measure out the required amount of 1:10 hydrochloric acid into a small beaker, add 5 ml. ferrous chloride solution and enough water to make 75 ml. Allow this to be drawn into *A* through *D*, cautiously at first lest too much foam be produced. If foam rises into the condenser, a drop of "antifoam" in the open end of *X* with a little water to wash it down will break up the bubbles. If this difficulty is anticipated, the addition may be made to the sample in the flask before the apparatus is connected. However, it is not necessary with many samples, and should not

be used as a routine measure, as it obscures the appearance of minute bubbles indicating incomplete decomposition of carbonate. As soon as foaming moderates, shake *B* to cause the liquid therein to swirl, in order that the crust of carbonate on the surface may be broken up and absorption hastened. Some judgment must be exercised here, as too sudden reduction in pressure within the apparatus as the result of rapid absorption of carbon dioxide may cause the foaming to become unmanageable, especially if the acid and soil have been warmed and no "antifoam" has been added. The apparatus should not be shaken violently—merely moved enough to cause the solution to swirl about. The clamp which grips the condenser jacket near its middle and the thick rubber tubing between readily permit sufficient movement.

When very vigorous evolution of gas has ceased, and most of it has been absorbed as shown by the appearance of the liquid in *B* and the reading of the manometer *G*, light the microburner *O* and adjust the flame to about 5 mm. height. Within a few seconds the mixture in *A* should be boiling vigorously and vapor condensing at about the middle of *C*. As long as considerable decomposition of carbonate continues, the acid and soil will boil quietly. When carbonate is practically all decomposed, violent bumping may be expected. To observe whether decomposition of carbonate is complete, remove the flame, shake the flask, and let it stand quiet for a few seconds. Observe closely whether any small bubbles continue to rise. If none appear within a half minute or so, all carbonate has been decomposed. This test is very delicate, because of the enormous expansion of bubbles in vacuo. Only in the case of samples ground in metal is there any danger of error. An inexperienced operator may possible mistake peculiar movements on the surface of the liquid, due to the effect of "antifoam" on surface tension, for bubbles quickly breaking. In both cases, however, the error will be on the safe side, so that there is no reason to fear incomplete decomposition. If much carbonate has been decomposed, the boiling should be continued about 8 minutes after this point, in order to be sure that all carbon dioxide has been driven into *B* and absorbed, the absorbent being agitated occasionally meanwhile. At the end of this time, the manometer *G* should read practically the same as it did before the acid was added, indicating that all carbon dioxide has been absorbed, that there has been no leak of air into the apparatus, and that the flow of water through the condenser has been sufficient to prevent accumulation of heat and rise in boiling temperature.

Before opening the apparatus, allow a little carbon dioxide-free water to be drawn in through *D* and *X* to wash the tube and condenser, then connect *D* to bead tower *I* and allow air to enter, slowly at first lest acid be thrown up into the condenser or alkali be drawn over from the tower. While the air is entering, agitate the absorbent in *B*. Disconnect *I*, *A*, and *B* in this order, and close the mouth of *B* with a one-hole rubber stopper plugged with a bit of heavy rod as soon as disconnected, first washing down the outside of the capillary tube extending into the flask with a spurt of water to remove any drops of the absorbent adhering.

Before beginning the titration, shake the liquid in *B*, remove the glass plug, and add the indicator. Insert the delivery tip of the burette filled with 0.1 *N* hydrochloric acid into the hole in the stopper and let the acid run in rather slowly with constant gentle swirling. As the excess of barium hydroxide is neutralized, reduce the rate at which the acid is added until each drop falls separately. As soon as each drop of acid produces a colorless zone in the liquid, discontinue the titration and shake the liquid vigorously, the hole in the stopper being closed with the bit of rod as before. If the amount of carbon dioxide absorbed has been considerable, so that the amount of acid required up to this point is much less than the blank, sufficient carbon dioxide-free water should be added to increase the volume of the solution to that of a blank determination. Neglect of this precaution may cause low results, because the high pH value at which the indicator turns requires an appreciable concentration of barium hydroxide. Continue the dropwise addition of acid from the burette until the indicator color is largely, but not entirely, discharged, then shake the flask again. Following this, some color should still remain, requiring 2 or 3 drops of acid to discharge completely. Deduct the burette reading from that of a blank determination conducted in the same way except that no sample is included, in order to get the volume of standard acid corresponding to the carbonate content of the sample.

Notes on the determination

Samples of peat soil and leaf mold are most troublesome, as the light and powdery material is prone to rise in dust when water is first added, and also foams persistently when acid is added. By the procedure described, these troubles can be overcome. Many of the precautions are not necessary with ordinary mineral soils.

During the latter part of the boiling period, after most of the carbon dioxide has been expelled, the mixture is likely to bump with great violence and be thrown high into the condenser. It is for this reason that the apparatus is made tall. Such a very violent bump always follows a period of perfect quiet, during which the solution is becoming slightly superheated. Gentle shaking will prevent violent bumping. At each bump, a loud click or "water hammer" is heard, leading the inexperienced operator to fear that the apparatus will collapse at a repetition. During the course of thousands of determinations, the author has had only two or three flasks cracked from this cause. In each instance a star-shaped crack appeared in the side of the flask, was noticed at once, and the determination saved by smearing grease upon the crack to stop the entrance of air. The only instance in which any part of the apparatus collapsed from air pressure was when a flat-bottomed flask was tried. Only spherical flasks can be depended upon to stand the pressure.

The condenser tube should not be constricted at the lower end, lest liquid thrown up and condensate be unable to return past the ascending vapor, and finally be thrown into *B*.

The pressure within the apparatus should be watched closely, as a rise indicates that the boiling temperature is rising, with risk of obtaining carbon dioxide from the decomposition of organic matter. Increase in pressure may be due to a leak, too large a flame, too warm condenser water, or ineffective absorption of carbon dioxide. If not due to leakage, the manometer reading should decrease when the flame is removed and the apparatus shaken. Poor quality, ill-fitting, or badly bored rubber stoppers are the chief cause of leaks; tubing has given little trouble.

If too large a flame is used, or the condenser water is warm, vapor may pass into *B* and condense there. Except that it is an indication that the apparatus is not properly managed, and that the temperature is rising, this does no harm. The vapor carries no hydrochloric acid, with the possible exception of a trace in spray.

The question of the optimum strength of acid to be used for the decomposition of the carbonates in a particular sample, or to be used for routine work, is a matter of judgment. One to fifty acid is entirely too weak for samples containing dolomitic limestone, and is probably weaker than necessary for routine use. Hydrochloric acid at 1:25 dilution with ferrous chloride will probably be more generally satisfactory. For samples containing dolomite, 1:10 acid is advised, always with ferrous chloride. If a determination has been started with weak acid and the continued evolution of carbon dioxide after a reasonable time indicates that dolomite is present, the strength of acid in the boiling flask is easily increased, best by the addition of 1:2 hydrochloric acid.

The time required for a determination will depend upon the nature of the carbonate mineral in the sample, the fineness to which it is ground, the strength of acid used for decomposition of carbonates, and the amount of agitation by boiling or shaking. If the carbonate is calcite, it will be decomposed in a short time, even with the 1:50 acid. If it is a very resistant limestone or dolomite, a 1:10 acid will be desirable. If a sample of this kind is ground to pass 100 mesh, decomposition should not require more than 45 minutes, and probably much less in most cases. The time required for complete expulsion of carbon dioxide from the acid and absorption in the barium hydroxide is about 8 minutes, when aided by several gentle shakings. Breaking the vacuum and dismantling the apparatus require about a minute. After some experience, the titration should not require more than 5 minutes. One can demonstrate in 7 or 8 minutes that a sample contains no carbonate, or only a trace, and complete a determination under the most favorable conditions in about 15 minutes. No properly prepared sample should require more than an hour for an accurate determination of the carbonate content. One man can handle three or four assemblies of apparatus, and with ordinary samples his time will be fully occupied.

Although the procedure described was developed for work with soils, there seems to be no reason why it should not be adapted to the determination of carbonates in other materials. For use with samples from which volatile acids other than carbonic are evolved under the conditions of the experiment, it

should be possible to neutralize the absorbent, filter and wash the precipitate, dissolve in excess standard acid, and titrate for an accurate determination of carbonate. Experiments have shown that by this procedure accurate results are obtained when hydrogen sulfide accompanies carbon dioxide. If the sample contains sulfite as well as carbonate, it should be possible to absorb both gases, add an oxidizing reagent and excess acid, and reabsorb carbon dioxide alone.[2]

SUMMARY

A simple apparatus and procedure for determination of carbonates in soil are described.

[2] Addendum, July 15, 1930.—Since this paper was accepted for publication, Dr. E. C. Shorey, of the soil fertility laboratory, Bureau of Soils, U. S. Department of Agriculture, has informed the author in a personal communication, dated July 10, that he believes the presence of uronic acids in soil to be a source of error in carbonate determinations. He has obtained evidence that substances of this class are of common occurrence in soils, and that they are more readily decarboxylated in the presence of quite dilute acids than has been realized.

The decarboxylation reaction

$$C_6H_{10}O_7 = C_5H_4O_2 + 3H_2O + CO_2$$

is quantitative, at least with respect to carbon dioxide, when uronic acids are boiled for a minimum of five hours with 12 per cent HCl. The furfuraldehyde is obtained in amount less than demanded by theory, but collection and determination of CO_2 evolved have been made the basis of a method for determination of uronic acids.* In a later paper,† it is stated that in the presence of very dilute acid the uronic acid is decomposed, but reversion products are formed which are very resistant to further decomposition, and the yield of CO_2 is no longer quantitative. In this case the course of the reaction is not a function linear with time, but proceeds at a rapidly diminishing rate. This seems particularly unfortunate, since it detracts from the value of an obvious means for correcting the error in carbonate determinations. With a constant rate of decomposition, it would be possible to make a second determination with twice the time allowed for the action of the acid, and any increase in CO_2 obtained during the doubled time would correspond to the blank to be applied to the first determination.

The authors of the latter paper state that with a decrease in temperature below 100°C. the rate of decarboxylation in the presence of dilute acid falls off rapidly, but present no data in support of the statement. As has already been mentioned in discussion of the data presented in table 1, increase in strength of acid and in time of action has been found to increase the amount of CO_2 obtained by the procedure described, even when ferrous chloride had been added to prevent an oxidizing action. In the case of three similar virgin surface soils containing considerable leaf mold and partly decomposed forest debris, a five-fold increase in strength of acid and a three-fold increase in time resulted in a constant increase in CO_2 obtained, about 0.08 m.e. per 100 gm. soil. This is probably the extent of the error due to decarboxylation from increase in severity of treatment. Even granting that these soils are entirely free from carbonates, and that the whole of the apparent carbonate content is due to decomposition of organic matter, the marked effect of ferrous chloride indicates that under the conditions of these experiments decarboxylation is a minor source of error compared to oxidation.

---

* Dickson, A. D., Otterson, Henry, and Link, K. P. 1930 A method for the determination of uronic acids. *Jour. Amer. Chem. Soc.* 52: 775–779.

† Link, K. P. and Niemann, Carl 1930 The action of weak mineral acids on uronic acids. *Jour. Amer. Chem. Soc.* 52: 2474–2480.

Carbon dioxide evolved from carbonates on boiling the sample with dilute hydrochloric acid containing ferrous chloride, below 30°C. in an evacuated apparatus, is absorbed in excess standard barium hydroxide solution under conditions ensuring complete absorption, and the excess barium hydroxide is titrated in the presence of the precipitated carbonate.

A study of the reasons for high results in the determination of carbonates in soil is reported. It is shown that oxidation of organic matter to carbon dioxide by reaction with manganese dioxide native to the soil and added acid may be a factor that is in many cases important even with the most dilute acid at room temperature. The addition of ferrous chloride to the acid used for decomposing soil carbonates is proposed as a remedy for this source of error.

As ferrous chloride does not entirely prevent evolution of carbon dioxide from soils containing no carbonates when treated with comparatively strong acid at higher temperature or for a longer time, the importance of conducting the determination at the lowest temperature and with the most dilute acid that can be used, consistent with complete decomposition of carbonates in a reasonable time, is stressed.

Special advantages claimed for the apparatus and procedure are greater simplicity, rapidity, and accuracy. Carbon dioxide from sources other than carbonate minerals is minimized, completion of decomposition can be recognized, and complete absorption ensured.

Application to determination of carbonates in materials other than soil is suggested and procedures outlined.

REFERENCES

(1) Association of Official Agricultural Chemists 1916 Report of the committee on editing tentative and official methods of analysis, p. 19. Baltimore, Md.

(2) Association of Official Agricultural Chemists 1925 Official and tentative methods of analysis, ed. 2, p. 22–24. Washington, D. C.

(3) Clark, N. A., and Collins, E .R. 1929 The gravimetric method for the determination of carbonates in soil. *Soil Sci.* 27: 407–414.

(4) Gaither, E. W. 1912 A new apparatus for the determination of carbon dioxide. *Jour. Indus. and Engin. Chem.* 4: 611–613.

(5) Hutchinson, H. B., and MacLennan, K. 1914 The determination of soil carbonates. *Jour. Agr. Sci.* 6: 323–327.

(6) Marr, F. S. 1910 Estimation of calcium carbonate in soils. *Jour. Agr. Sci.* 3 (pt. 2): 155–160.

(7) Robinson, W. O. 1929 Detection and significance of manganese dioxide in soil. *Soil Sci.* 27: 335–350.

(8) Schollenberger, C. J. 1916 Estimation of carbon dioxide as barium carbonate applied to the Marr method for the determination of carbonates in soil. *Jour. Indus. and Engin. Chem.* 8: 427–428.

(9) Schollenberger, C. J. 1928 Thymolphthalein as indicator for titrimetric estimation of carbon dioxide. *Indus. and Engin. Chem.* 20: 1101.

(10) Truog, E. 1915 Methods for the determination of carbon dioxide and a new form of absorption tower adapted to the titrimetric method. *Jour. Indus. and Engin. Chem.* 7: 1045–1049.